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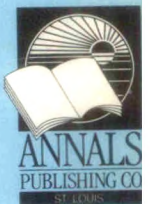
ENDOSCOPIC MANAGEMENT OF A
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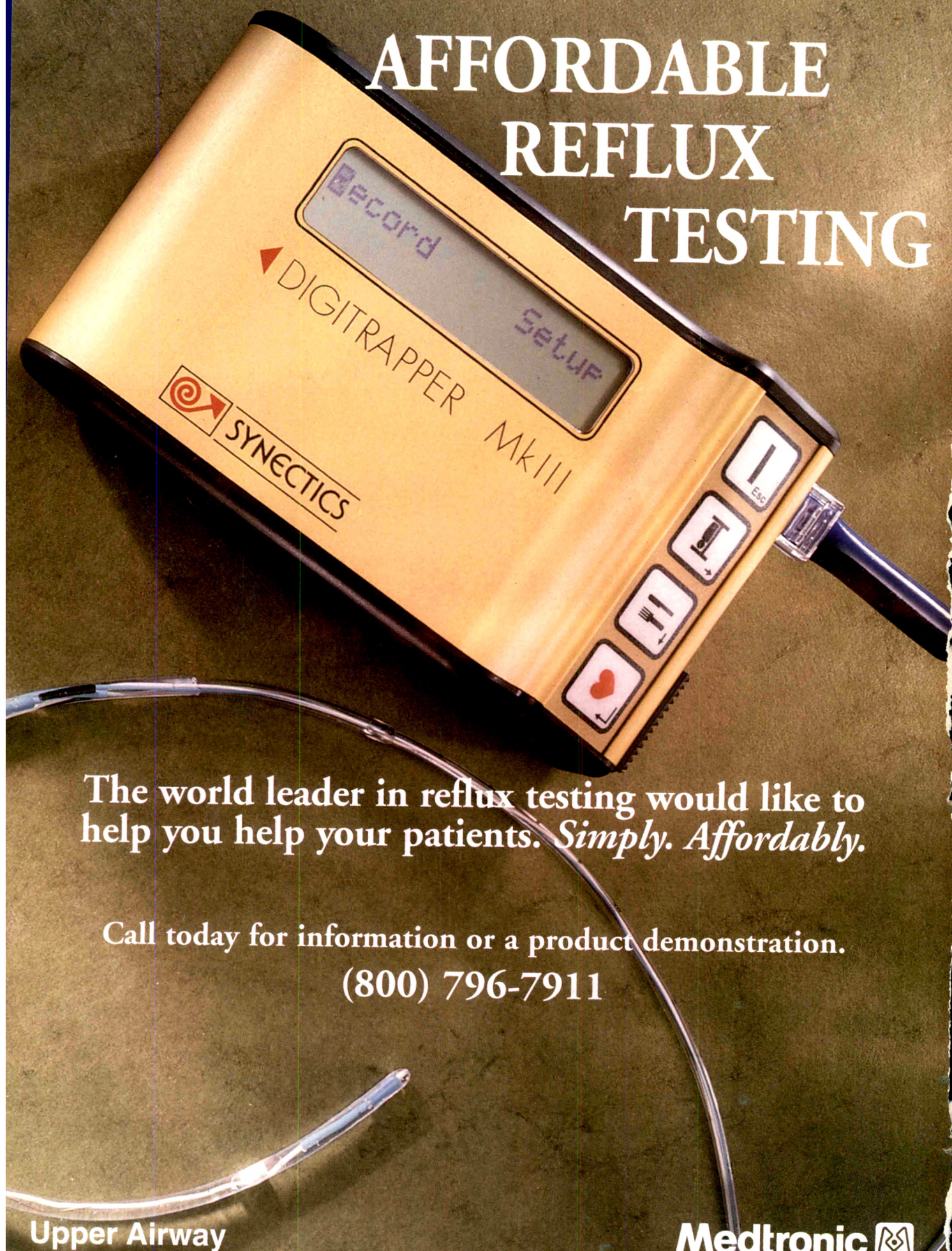
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RECURRENT PLEOMORPHIC ADENOMA
OF THE PAROTID GLAND INVOLVING THE
OSSEOUS EXTERNAL AUDITORY CANAL

INTERACTION BETWEEN ORAL ALPHA-
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REINKE'S EDEMA: PHONATORY MECHANISMS AND MANAGEMENT STRATEGIES

STEVEN M. ZEITELS, MD
GLENN W. BUNTING, MS

ROBERT E. HILLMAN, PHD
TRACI VAUGHN

BOSTON, MASSACHUSETTS

Reinke's edema (RE) has been associated typically with smoking and sometimes with vocal abuse, but aspects of the pathophysiology of RE remain unclear. To gain new insights into phonatory mechanisms associated with RE pathophysiology, we used an integrated battery of objective vocal function tests to analyze 20 patients (19 women) who underwent phonomicrosurgical resection. Preoperative stroboscopic examinations demonstrated that the superficial lamina propria is distended primarily on the superior vocal fold surface. Acoustically, these individuals have an abnormally low average speaking fundamental frequency (123 Hz), and they generate abnormally high average subglottal pressures (9.7 cm H₂O). The presence of elevated aerodynamic driving pressures reflects difficulties in producing vocal fold vibration that are most likely the result of mass loading associated with RE, and possibly vocal hyperfunction. Furthermore, it is hypothesized that in the environment of chronic glottal mucositis secondary to smoking and reflux, the cephalad force on the vocal folds by the subglottal driving pressure contributes to the superior distention of the superficial lamina propria. Surgical reduction of the volume of the superficial lamina propria resulted in a significant elevation in fundamental frequency (154 Hz) and improvement in perturbation measures. In almost all instances, both the clinician and the patient perceived the voice as improved. However, these patients continued to generate elevated subglottal pressure (probably a sign of persistent hyperfunction) that was accompanied by visually observed supraglottal strain despite the normal-sized vocal folds. This finding suggests that persistent hyperfunctional vocal behaviors may contribute to postsurgical RE recurrence if therapeutic strategies are not instituted to modify such behavior.

KEY WORDS — laryngoscopy, laser, microlaryngoscopy, microsurgery, phonosurgery, polypoid corditis, Reinke's edema, stroboscopy, vocal cord, vocal fold, voice.

INTRODUCTION

Reinke's edema (Fig 1A,B) has been observed as a clinical entity since the introduction of the conventional laryngoscope and the origin of laryngology. Turck¹ depicted this condition in his clinical atlas in 1860 and referred to it as chronic inflammation of the vocal cords. In the 1890s, Hajek² and then Reinke^{3,4} performed infusion experiments in the larynx to simulate membranous edema and to understand the mechanisms of laryngeal airway obstruction. Hajek⁵ credited Reinke with identifying the subepithelial compartment in the vocal fold that is superficial to the vocal ligament, bounded anteriorly by Broyle's ligament, and limited posteriorly by the arytenoid. Hirano⁶ has identified this compartment as the superficial lamina propria. Zeitels⁷ noted that this compartment underlies the vibratory epithelium of the musculo-membranous vocal fold, which can be identified caudally by the inferior arcuate line. Over time, extensive chronic swelling of the superficial lamina propria (Fig 1A,B) has become known as Reinke's edema.

Reinke's edema has been associated usually with smoking,^{8,9} frequently with vocal abuse,^{10,11} and oc-

asionally with reflux¹² (also J. A. Koufman, unpublished observations, 1995). There is a commonality to the first and third factors, in that there is a caustic exposure to the glottal mucosal membranes. Although vocal abuse has been cited descriptively as an etiologic factor in the development of Reinke's edema for over 50 years, the actual role of vocal hyperfunction in the development of this disorder has remained obscure. Previous investigations of vocal function in patients with Reinke's edema have utilized only perceptual and acoustic analyses.¹³⁻¹⁵ In the present study, a battery of objective measures (stroboscopic, acoustic, and aerodynamic) in conjunction with clinically based perceptual judgments was employed to study a cohort of patients with Reinke's edema before and after phonomicrosurgical resection. It was hoped that the data from these measures would help elucidate the impact of Reinke's edema on laryngeal sound production and begin to provide insights into underlying vocal mechanisms (eg, vocal hyperfunction) that could potentially be associated with the development of this disorder.

MATERIALS AND METHODS

Individuals were selected for this study who pre-

From the Department of Otolaryngology, Harvard Medical School (Zeitels, Hillman, Vaughn), and the Department of Otolaryngology, Massachusetts Eye and Ear Infirmary (Zeitels, Hillman, Bunting), Boston, Massachusetts.

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CORRESPONDENCE — Steven M. Zeitels, MD, Dept of Otolaryngology, Massachusetts Eye and Ear Infirmary, 243 Charles St, Boston, MA 02114.

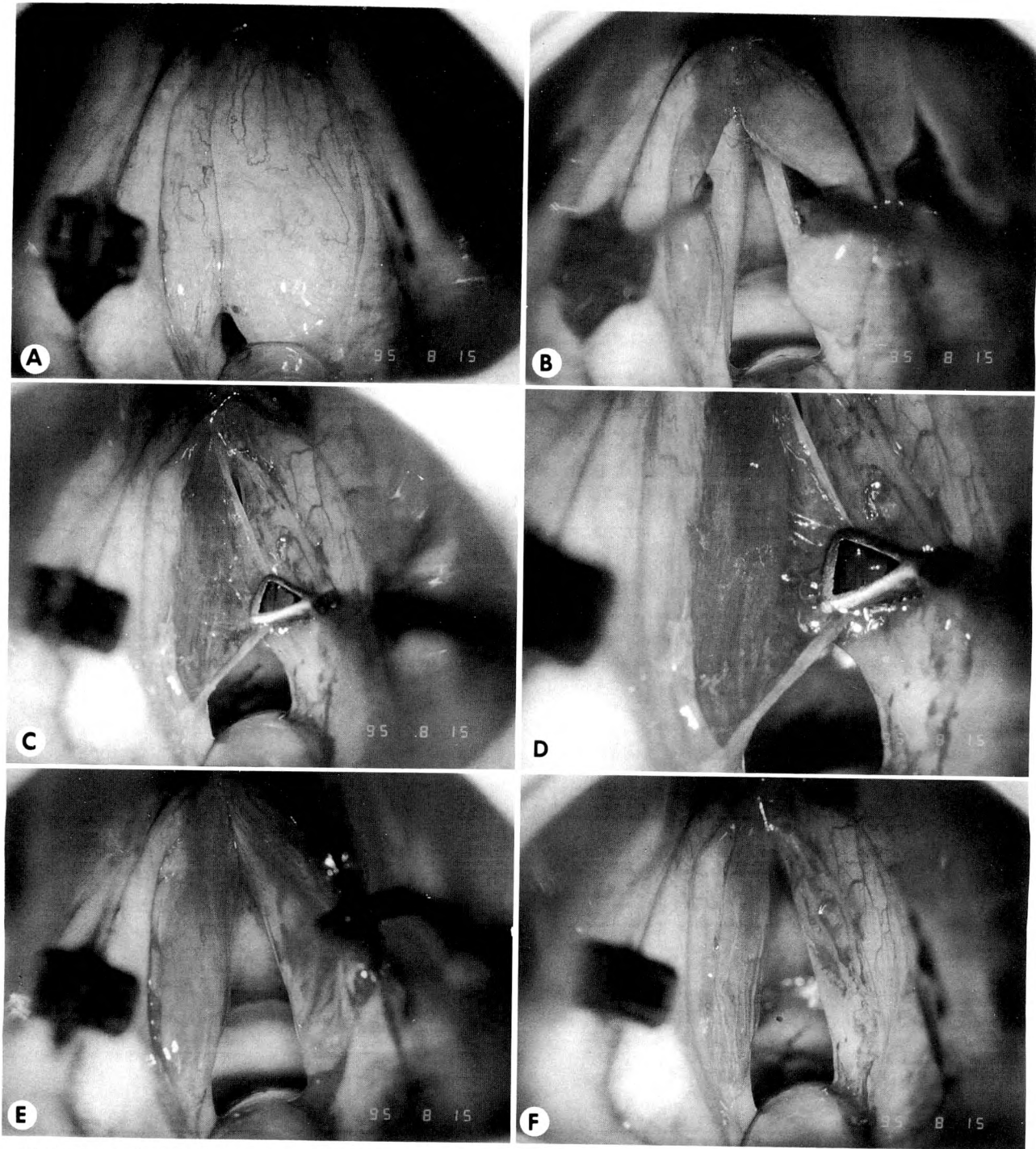


Fig 1. Phonomicrosurgical resection. A) Massive asymmetric Reinke's edema. B) Edema, which is situated on superior surface of vocal folds, is retracted laterally. C) Superiorly based microflap is raised to facilitate suctioning of myxoid superficial lamina propria to desired contour. D) Higher magnification of C, demonstrating reduced volume of superficial lamina propria and translucent epithelium. E) After suctioning of excessive superficial lamina propria in right vocal fold, epithelium is redraped and retracted laterally with curved dissector to estimate amount of redundant epithelium that requires resection. F) Final result after phonomicrosurgical resection, which demonstrates normal vocal fold contours and epithelial edges that appose well.

sented to the surgeon with clinical findings of Reinke's edema (Fig 1A,B) and complaints of hoarseness for which the patient elected surgical intervention. All patients underwent at least 1 month of preoperative antireflux management, which consisted of strict diet and behavior modification, as well as medical man-

agement with either omeprazole or an H₂ antagonist. All patients underwent a preoperative consultation with a speech-language pathologist in which objective measures of vocal function were obtained along with preoperative counseling (see below). Smoking histories were obtained before and after the phono-

microsurgical intervention.

SURGICAL TECHNIQUE

The technique for surgical exposure has been previously described^{7,16} (Fig 1A,B). An initial incision was made on the superior surface of the vocal fold. The incision was made with an upturned microscissors or a microspot carbon dioxide laser.¹⁷ The latter instrument was chosen when prominent subepithelial vascularity was noted. Subsequently, an epithelial microflap was raised with a Bouchayer dissector (Instrumentarium Co). The myxoid stroma in the superficial lamina propria was then suctioned in a fashion similar to that described by Hirano et al.¹⁸ During the suctioning, great care was taken to leave a normal or slightly increased volume of superficial lamina propria adjacent to the medial lamina propria and to avoid traumatizing the remaining superficial lamina propria and vocal ligament (Fig 1C,D). On occasion, the myxoid stroma of the superficial lamina propria was so viscous that it required excision with cold instrumentation. After the superficial lamina propria was reduced in volume to the desired contour, the redundant epithelium was redraped (Fig 1E) to determine the volume of epithelial resection that would allow for apposition of the mucosal edges (Fig 1F). Any keratotic epithelium that was suspicious for atypia or microinvasive cancer was resected en bloc, mounted on a carrier, and serially sectioned for whole mount analysis. During the procedure, hemostasis was controlled by delicately applying 1/10,000 epinephrine-saline solution-soaked cotton to the bleeding surface. At the conclusion of the surgery, the vocal folds were sprayed with plain 2% lidocaine to avoid laryngospasm during emergence from anesthesia.

Postoperatively, patients were asked to remain on strict voice rest for 10 days. Subsequently, patients began modified voice rest under the guidance of a speech-language pathologist until they resumed normal vocal activities at about 3 weeks. They were continued on a rigorous antismoking and antireflux protocol postoperatively.

VOICE ASSESSMENTS

All patients underwent complete preoperative and postoperative voice assessments that included the following.

Visual (Videolaryngoscopy With Stroboscopy). Standard transoral rigid (Kay Elemetrics 70°) or transnasal flexible (Olympus ENFP-3) videolaryngoscopy with stroboscopy was performed as each patient sustained /i/ vowels at comfortable (average) pitch and loudness levels. These examinations were recorded on videotape (SVHS [super video home system] format)

and played back at weekly case conferences where they were rated by otolaryngologists and speech-language pathologists (ie, the "voice team") via group discussion and consensus. The basic clinical rating scheme includes 1) vocal fold mobility (adduction and abduction), 2) integrity of the epithelial surface and contour of the vocal fold, 3) glottal closure pattern, 4) mucosal wave activity, and 5) supraglottal compression (vestibular fold and anterior-posterior).

Videoscopic assessment before stroboscopic analysis was used to stage the polypoid changes. Similar to that described by Yonekawa,¹⁴ a grading system was used that assessed the severity of the Reinke's edema. The grade was determined on inspiration, with a normal tidal volume and abducted arytenoids: in grade I, contact of the vocal folds was confined to the anterior third of the musculomembranous vocal folds; in grade II, contact of the vocal folds was confined to the anterior two thirds of the musculomembranous vocal folds; and in grade III, contact of the vocal folds extended to the posterior third of the musculomembranous vocal folds. The disease was also characterized as being symmetric or asymmetric. Asymmetry was defined as a greater than 30% difference in size of the folds as judged by the surgeon at the time of microlaryngoscopy.

A qualitative assessment of the severity of laryngopharyngeal reflux was done, based on endoscopic examination performed preoperatively and postoperatively, at the time of the final vocal analysis. Severity was graded from I to III on the basis of history and physical examination. Specific indicators on the physical examination for reflux included the presence and magnitude of erythema and edema of the membranes of the larynx and hypopharynx. The character, volume, and location of secretions in these areas was also noted, as well as the presence of interarytenoid hyperplastic epithelium and pachyderma laryngis. Also, symptoms such as throat clearing, globus sensation, indigestion, heartburn, dysphagia, and sour taste were recorded.

Subjective Judgments of Voice Quality. Vocal assessments were carried out by speech-language pathologists with an average of 5 years of experience in evaluating and treating patients with voice disorders. As part of the assessment, clinicians made subjective judgments of vocal quality parameters, including 1) pitch (adequate, too high, too low), 2) loudness (adequate, too loud, too soft), and 3) presence of hoarseness, straining, and breathiness (none, mild, moderate, severe). Each patient was also asked how his or her voice after surgery compared to how it was before surgery (same, worse, better).

Acoustic Assessment. Each patient was seated in a

sound-treated room (IAC booth) and wore a headband device that suspended an electret condenser microphone a constant distance of 15 cm from the lips. The output of the microphone was fed to a preamplifier and then recorded on digital audiotape. A calibration tone of known intensity (in decibels sound pressure level [SPL]) was recorded prior to each patient evaluation and used as a reference for acoustic analysis. Recordings were made as each patient performed the following tasks: 1) read a standard passage (the Rainbow Passage), 2) sustained the vowel /a/ three times, holding pitch and loudness as "steady" (constant) as possible for at least 3 seconds, and 3) sustained the /a/ vowel three times at a comfortable level for as long as possible on one breath.

The audio recordings of the reading passage (10-kHz sampling rate) and vowels (20-kHz sampling rate) for each patient were digitized and processed on a 486/33 PC workstation with software that is commercially available (Computer Speech Lab, Kay Elemetrics, Inc). The reading passage was analyzed to yield measures of average fundamental frequency (F0) and intensity (in decibels). The steadiest 1-second segment from the middle portion of the "best" (ie, perceptually judged to be the steadiest and to have the best quality) of the /a/ vowels produced for 3 seconds was analyzed to yield percent frequency perturbation (jitter) and percent intensity perturbation (shimmer). Maximum phonation time was determined from the longest-sustained /a/ vowel.

Aerodynamic Testing. Aerodynamic testing was done with an Aerophone system (Kay Elemetrics, Inc). Patients produced strings of "pah" syllables at a comfortable (normal) level while the following measures were obtained: 1) oral airflow measured with a pneumotachygraph mounted in a face mask and 2) intraoral air pressure measured with a translabially placed catheter connected to a pressure transducer. The flow and pressure signals were digitized and analyzed on the Aerophone system to yield indirect estimates of average glottal airflow rate (in liters per second) and subglottal air pressure (in centimeters of water).

STUDY GROUPS

There were 20 patients reviewed in this series, including 1 patient who had mild Reinke's edema and keratosis with atypia on one vocal fold. This patient underwent phonomicrosurgical resection of the atypia without formal resection of the mild polypoid corditis and was, therefore, not included in the analysis. Eighteen of the 19 patients that could be evaluated were women. Since the lone male displayed complete recurrence of Reinke's edema, and because many voice measures are gender-dependent (F0, subglottal

pressure, etc), this individual was excluded from group-based comparisons. The presurgery group (PRE) comprised all 18 women. Postsurgery group 1 (POST-1) comprised 14 women in whom the post-surgical assessment did not reveal any overt signs of polypoid recurrence. Postsurgery group 2 (POST-2) was made up of 4 women who displayed obvious signs of mild polypoid recurrence. Because of the small number of subjects in POST-2, formal statistical analyses (*t*-tests for paired comparisons) were limited to comparisons between PRE and POST-1. The postoperative analyses were typically scheduled for 2 to 4 months after the procedure. For a variety of reasons (typically, compliance), a number of individuals were studied 6 to 30 months after surgery.

RESULTS

PREOPERATIVE ENDOSCOPIC ANALYSIS WITH STROBOSCOPY

Fifteen of the 19 patients had bilateral disease, and both vocal folds were operated on at one surgical encounter. Four of the 19 patients had unilateral disease, and 1 of these patients had a 20-year history of paralysis of the contralateral vocal fold. This latter individual was the sole patient that did not have a smoking history. There was 1 male patient in this series, and he had unilateral disease.

Videoendoscopic evaluation revealed that the Reinke's edema was located primarily on the superior surface of the vocal folds. It was not uncommon to observe some degree of asymmetry to the magnitude of the edema. Of the 15 patients with bilateral disease, 5 had remarkable asymmetry in the volume of the vocal folds. Therefore, 10 of the 19 patients presented with bilateral symmetric disease. Seven of these 10 had grade III severity (Table 1). All 5 patients with an asymmetric presentation had grade III severity. The 4 patients with unilateral disease had grade I or II severity.

Endoscopic examination also revealed that 17 of the 19 patients had vestibular fold compression and 18 of the 19 had anteroposterior supraglottal compression. All of the patients were judged to have accompanying laryngopharyngeal reflux (based on the subjective assessment scheme). It was severe in 11 of the 19 and moderate in 8 (Table 2).

The stroboscopic examinations more precisely localized the edema of the superficial lamina propria to be situated on the superior surface of the vocal fold (Fig 1B). General assessment measures of mucosal wave propagation were felt to be unsuitable for examining the highly irregular consistency and volume of vocal folds with extensive polypoid corditis. Although a number of individuals demonstrated hy-

TABLE 1. SEVERITY OF REINKE'S EDEMA

Disease Presentation	Grade of Edema			Total
	I	II	III	
Symmetric	0/3	3/0	7/0	10/3
Asymmetric	0/1	0/0	5/0	5/1
Unilateral	1/0	3/1	0/0	4/1
Total	1/4	6/1	12/0	19/5

Data are preoperative/postoperative numbers of patients. Each of following grades was determined on inspiration, with normal tidal volume and abducted arytenoids: grade I — contact of vocal folds was confined to anterior third of musculomembranous vocal folds; grade II — contact was confined to anterior two thirds; grade III — contact extended to posterior third.

perdynamic waves, this finding was dependent on the variable viscoelastic distensibility of the edematous superficial lamina propria. Some edematous folds were not pliable, and produced a highly aberrant mucosal wave as judged by conventional standards. Also, it was not infrequent for the larger of a pair of asymmetric folds to impair the mucosal wave on the contralateral side by means of encroachment. A new assessment protocol is being designed to address these difficulties in assessment.

POSTOPERATIVE ENDOSCOPIC ANALYSIS WITH STROBOSCOPY

Of the 19 patients, 5 had recurrence of the Reinke's edema at the time of the voice analysis. All 5 patients had resumed their smoking habit. Due to poor compliance in returning for analysis, these individuals were studied more than 6 months after surgery (16 months on average). Four of the 5 patients (Table 1) showed improvement in the appearance of the glottis despite evidence of mild recurrence of the polypoid changes. The sole male patient, who had unilateral disease, was studied 15 months after surgery and his state appeared identical to his preoperative one. There were 3 other patients who were analyzed an average of 2 years after surgery; they had no recurrence and were not smoking. None of the patients who had bilateral disease developed an anterior web, despite bilateral surgery at one surgical encounter.

Endoscopic examination also revealed that there was improvement in supraglottal strain. In POST-1 (nonrecurrence), vestibular fold compression decreased to 7 of the 14 patients, and anteroposterior compression decreased to 10 of the 14 patients. As was not unexpected in POST-2 (partial recurrence), supraglottal strain patterns persisted, and in fact, both types were observed in 3 of the 4 patients. By the time the patients were analyzed postoperatively, they had been managed for reflux for a minimum of 3 months, and most showed improvement. Postoperatively, there was generalized improvement in reflux according to history and endoscopic observations of the larynx

TABLE 2. SEVERITY OF REFLUX LARYNGOPHARYNGITIS

Disease Presentation	Grade of Reflux			Total
	I	II	III	
Symmetric	0/0	3/9	7/1	10/10
Asymmetric	0/2	3/3	2/0	5/5
Unilateral	0/0	2/4	2/0	4/4
Total	0/2	8/16	11/1	19/19

Data are preoperative/postoperative numbers of patients. Grades I through III indicate increasing severity (most severe = III) of reflux.

and laryngopharynx (Table 2).

Postoperative stroboscopic examinations revealed that in POST-1, 11 of the 14 patients had normal mucosal waves (amplitude, magnitude, and phase symmetry), including 4 of the 5 patients who presented with unilateral disease. Three of the 14 patients had mild impairment of mucosal wave propagation. Of the patients who showed recurrence (POST-2), mucosal wave propagation was normal in 3 of the 4.

VOICE QUALITY

Results for clinician-based judgments of voice quality are summarized in Fig 2. The abscissa in Fig 2 represents the number of rating categories that patients' voices changed in comparing the presurgery

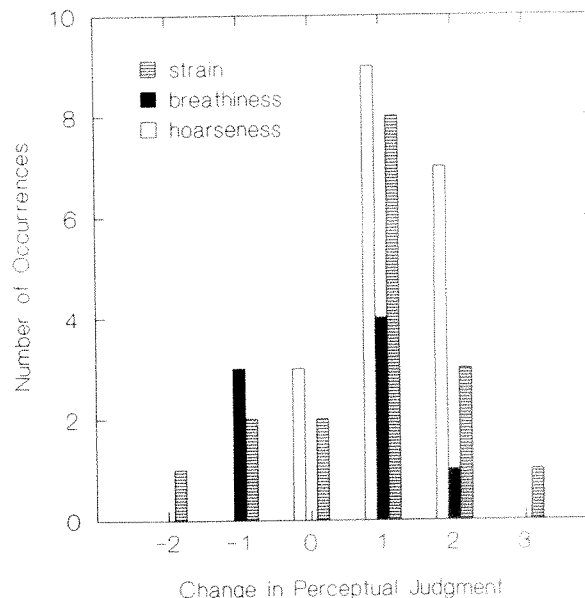


Fig 2. Histogram showing cumulated tallies of changes in clinician-based judgments of voice quality. Abscissa (Change in Perceptual Judgment) represents number of rating categories that patients' voices changed in comparing presurgical versus postsurgical evaluations, while ordinate (Number of Occurrences) shows total number of patients who displayed each type of change. Improvements in quality dimensions are reflected in positive numbers (eg, severe to mild is +2), while deteriorations are shown by negative numbers (eg, mild to severe is -2), and no change is 0. Ratings were cumulated across presurgical and postsurgical assessments for all 19 patients.

TABLE 3. SUMMARY OF RESULTS FOR ACOUSTIC AND AERODYNAMIC MEASURES

Measure	PRE (N = 18)	POST-1 (N = 14)	POST-2 (N = 4)	Normal Values	p
Fundamental frequency for reading (Hz)	123 ± 20	154 ± 18	129 ± 23	180-230	<.001
Sound pressure level for reading (dB)	65.8 ± 7.7	64.9 ± 3.3	66.8 ± 2.5	67-73	.680
Maximum vowel phonation (s)	11.3 ± 7.4	11.9 ± 5.9	11.0 ± 8.9	12.0	.816
Jitter (%)	2.03 ± 2.32	1.56 ± 1.40	2.93 ± 2.21	1.04	.285
Shimmer (%)	7.02 ± 3.24	4.68 ± 1.85	10.91 ± 7.91	3.81	.001
Pressure (cm H ₂ O)					
Comfortable voice	9.7 ± 3.8	9.1 ± 2.2	9.2 ± 0.9	7.8	.650
Loud voice	14.9 ± 4.8	14.2 ± 5.3	16.7 ± 3.6	11.9	.646
Flow rate (L/s)					
Comfortable voice	0.23 ± 0.13	0.23 ± 0.11	0.22 ± 0.04	0.07-0.33	.933
Loud voice	0.26 ± 0.18	0.22 ± 0.11	0.16 ± 0.08	0.07-0.37	.523

Data are average values and associated standard deviations for each study group, normative data in terms of ranges or threshold values, and p values from *t*-tests comparing PRE (presurgery) and POST-1 (postsurgery with no polypoid recurrence). POST-2 — postsurgery with mild polypoid recurrence.

versus postsurgery evaluations, while the ordinate shows the total number of patients who displayed each type of change. For example, a reduction (improvement) from severe (presurgery) to mild (postsurgery) hoarseness would be reflected by a +2, while a worsening from mild to severe would be coded as -2. Ratings were cumulated across the presurgical and postsurgical assessments for all 19 patients.

There are a total of 44 comparisons plotted in Fig 2. A clear majority of comparisons (33 of the 44 or 75%) reflected postsurgical improvements in voice quality, with most improvements being accounted for by reductions in hoarseness (16) and straining (12), and a lesser number by reduced breathiness (5). Only a small number of comparisons reflected postsurgical deterioration (6 of the 44 or 14%) or no change (5 of the 44 or 11%) in voice quality.

The speech-language pathologists judged vocal pitch to be too low in 17 of the 19 patients presurgically; of the remaining 2 patients, 1 had adequate pitch and 1 had pitch that was too high (accompanying vocal fold paralysis). Fifteen of the 19 were considered to still have abnormally low vocal pitch postsurgically; all 4 of the remaining patients had adequate pitch. In terms of loudness, 12 of the 19 patients were judged to have adequate vocal loudness presurgically; of the remaining 7 patients, 5 voices were too soft and 2 were too loud. Fifteen of the 19 displayed adequate loudness postsurgically; of the remaining 4 patients, 2 voices were too soft and 2 were too loud.

In terms of patients' self-assessments, 18 of the 19 patients reported that their voices were better following surgery as compared to before surgery. Only 1 patient reported that her voice was the same following surgery.

ACOUSTIC RESULTS

Table 3 provides a summary of the results for the acoustic and aerodynamic measures. Initially, acoustic and aerodynamic data were stratified by severity classification, ie, type II versus type III. However, there were no significant differences between type II and type III groups of patients (eg, average F₀ for type II was 122 Hz, and for type III, 119 Hz), so data were collapsed across presurgical severity types. Shown are the average values and associated standard deviations for each study group, normative data in terms of ranges or threshold values, and p values from *t*-tests comparing PRE (presurgery) and POST-1 (postsurgery with no recurrence).

The average F₀ prior to surgery (PRE) fell well below the lower end of the normal range for adult females (180 Hz). The postsurgery group that had no recurrence (POST-1) displayed a significant (*p* < .001) increase of 31 Hz in average F₀ as compared to PRE. However, the F₀ value for POST-1 still fell below the normal range. The postsurgery group with recurrence (POST-2) displayed an average F₀ value that approximated that of the presurgery group (PRE). Average SPL values for reading were borderline low for all three study groups as compared to the normal range. There was no significant difference in average SPL between the PRE and POST-1 groups.

Average perturbation values for sustained vowels (percent jitter and shimmer) for all three study groups were well above the normal threshold values used by the Computer Speech Lab analysis system. There was, however, a significant (*p* = .001) postsurgical decrease in average shimmer for POST-1 as compared to PRE. Jitter also decreased in the POST-1 group versus the PRE group, but the difference was not statistically significant (*p* = .285). The group of subjects with recurrence (POST-2) displayed the

highest average perturbation values.

Average maximum phonation time values were borderline low for all three study groups. There was no significant difference in average maximum phonation time between the PRE and POST-1 groups.

AERODYNAMIC RESULTS

Average subglottal pressure values in both comfortable and loud voice exceeded the upper end of the normal range for all three study groups, and there was no significant difference in average subglottal pressure between the PRE and POST-1 groups. Average airflow rates for both comfortable and loud phonation fell within the normal range for all three study groups, and there was no significant difference between the PRE and POST-1 groups.

DISCUSSION

PRESENTATION OF REINKE'S EDEMA

There have been a number of terms used to denote the clinical entity we recognize as Reinke's edema. These terms include polypoid corditis,¹⁰ polypoid laryngitis, polypoid degeneration,¹⁹ and chronic hypertrophic laryngitis.¹¹ The population in this series was overwhelmingly female: 95%. This percentage is virtually identical to that of Fritzell and Hertegard,²⁰ who reported a 94% incidence of females in their series of 140 patients. Most series do report a much higher percentage of women with Reinke's edema; however, this probably reflects the social unacceptability of the voice quality for females, which leads to their seeking medical attention. There may also be an endocrinologic aberration that has not as yet been elucidated. The decreased F₀ that accompanies the polypoid vocal folds is stigmatizing for women and not typically a problem for men.

A strong association between smoking and Reinke's edema is uniformly accepted. Laryngopharyngeal reflux was cited recently as a potential cofactor in the development of Reinke's edema.¹² Our clinical observations, which were based on the endoscopic examination as well as history, confirmed Koufman's unpublished finding that 5 of 5 patients with Reinke's edema had laryngopharyngeal reflux and abnormal results on pH studies. It is reasonable to speculate that caustic mucosal exposure from smoking and reflux could cause increased susceptibility of the glottal mucosa to injury and subsequent lesion formation.

OBJECTIVE AND PERCEPTUAL EVALUATIONS

Endoscopic analysis revealed that 14 of the 19 patients did not demonstrate recurrence of the Reinke's edema in the early postoperative period (<6 months). Of the 8 patients that were vocally analyzed more

than 6 months after surgery, 5 demonstrated evidence of recurrence of the Reinke's edema, and all 5 had resumed smoking. However, 4 of the 5 remained improved over the preoperative state. The other 3 of the 8 patients with a more than 6-month postoperative analysis did not resume smoking and showed no evidence of recurrence of Reinke's edema. There were a number of other endoscopic and stroboscopic observations that reflected improvement in the larynges of these patients with Reinke's edema. Supraglottal strain and compression were reduced but still present in many patients. Also, mucosal wave propagation improved in most. In fact, 5 patients who could not be evaluated preoperatively by conventional grading systems became normal postoperatively.

Results from acoustic analysis of average speaking F₀ and clinician-based perceptual judgments are in general agreement with previous investigations of Reinke's edema. To briefly summarize, the abnormally low presurgical vocal F₀ in women with Reinke's edema increased significantly following surgery (average increase of 31 Hz), but did not attain a normal level for nonsmokers. This finding has been observed by previous investigators.^{14,15,21} In particular, the average postsurgical F₀ value (154 Hz) for the group with no recurrence (POST-1) is in close agreement with data reported by Bennett et al²¹ (150 to 165 Hz), who cite previous work on smokers' voices^{22,23} and point out that such values "more closely resembled the 165 Hz group value that has been reported for women who smoke, but who do not have polypoid degeneration."^{21(p528)} This is somewhat at odds with Fritzell et al,¹³ who reported that many of their surgically treated female Reinke's edema patients produced an average speaking F₀ that fell within normal limits. Findings of an improvement in perceived voice quality (eg, reduction in hoarseness) following surgical treatment of Reinke's edema are also in agreement with previous reports.^{13-15,21}

Additional acoustic measures of perturbation for sustained vowels were generally supportive of clinicians' perceptions that voice quality improved following surgery; ie, there was a significant postsurgical reduction in shimmer, as well as a drop in average jitter that did not reach statistical significance. The perceptual and acoustic data (including F₀ results) are also in concert with perceptions of the vast majority of patients (18 of 19), who reported that their voices were "better" following surgery.

Average airflow rates for all three study groups fell within normal limits and thus did not reflect abnormality in vocal function. Average airflow rate is a rather variable measure, even in repeated recordings of normal speakers, and is only sensitive to relatively

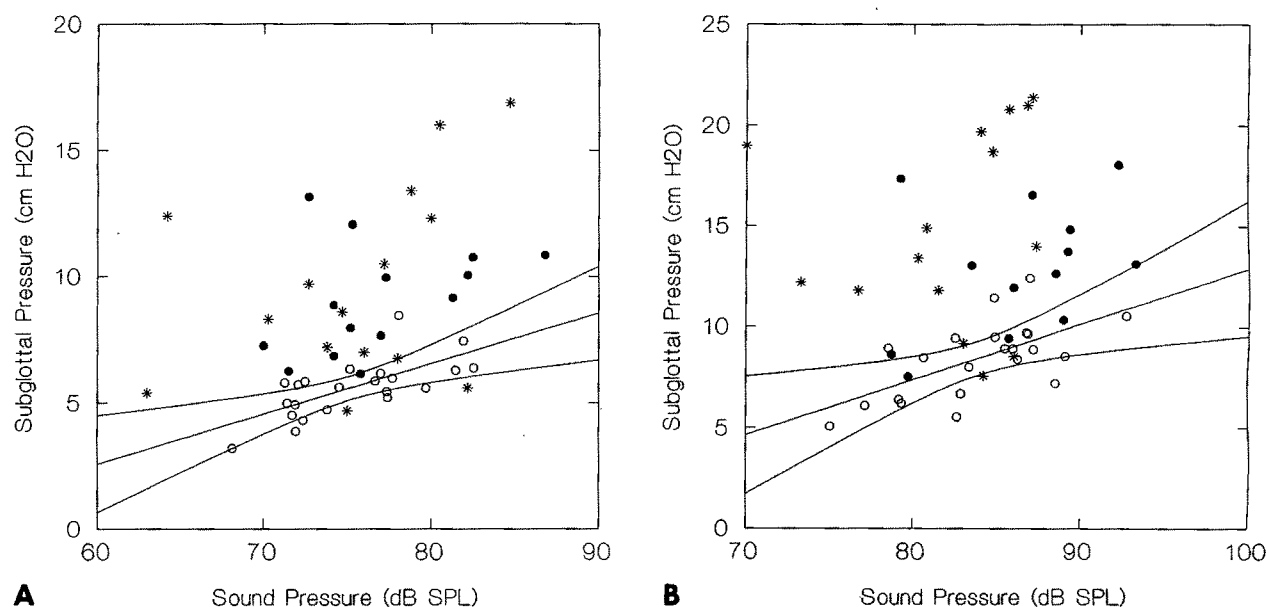


Fig 3. Plots of sound pressure level (SPL) versus subglottal air pressure for presurgical (asterisks) and postsurgical (solid circles) conditions. Also shown are data for group of normal women (open circles) with associated linear regression line and 90% confidence interval. A) At comfortable voice intensity. B) At loud voice.

dramatic changes in mechanisms related to glottal closure.²⁴ Altered glottal closure was not a major factor in presurgical or postsurgical Reinke's edema patients.

Figure 3 shows plots of SPL versus subglottal pressure for the comfortable and loud voice conditions. Each Figure displays data for both the PRE (asterisks) and POST-1 (solid circles) groups. Also shown on each plot are data for a group of normal women (open circles) with associated linear regression lines and 90% confidence intervals.²⁵ Results showed that presurgical and postsurgical polypoid patients had somewhat higher-than-normal average subglottal pressures. However, given the strong relationship between subglottal pressure and SPL, it is more meaningful to assess estimates of subglottal pressure relative to SPL.²⁴ Thus, Fig 3 even more clearly demonstrates that a majority of both presurgical (PRE) and postsurgical (POST-1) patients were generating much higher subglottal pressures than are required by normal speakers to produce an equivalent SPL. The fact that such higher pressures were not associated with a proportionally higher SPL reflects a reduction in the efficiency of both the presurgical and postsurgical vocal mechanisms of patients with polypoid corditis.

As part of a previous investigation of phonatory function in hyperfunction-related vocal fold lesions, Hillman et al²⁶ examined 2 women with Reinke's edema and found that their subglottal pressures were within normal limits. Given the results from the present study, it now appears that these earlier pres-

sure data are not typical of patients with Reinke's edema. Note that the current data set also contains subglottal pressure values for a few subjects that fall within or close to the normal range, but that these are the exceptions.

Abnormally high subglottal pressures have been associated with a variety of hyperfunction-related voice disorders,^{26,27} including vocal nodules, polyps, and functional dysphonias. There is also preliminary evidence that subglottal pressures remain elevated following surgical removal of hyperfunction-related vocal fold lesions.²⁷ This latter observation is in line with results from the present study that showed a persistence of elevated subglottal pressures postsurgically. One possible interpretation that has been offered previously for such observations is that elevated postsurgical pressures reflect the persistence of presurgical vocal habits; ie, vocal hyperfunction causes the vocal folds to be abnormally stiff and tense and increases the force of adduction, thus requiring higher driving pressures for phonation. In addition, hyperfunction-related vestibular fold compression may squeeze the edematous vocal folds²⁸ and thereby decrease their viscoelastic pliability and contribute to oscillation abnormalities. Even though there was a postsurgical reduction of supraglottal compressive behaviors, many patients still displayed consistent ventricular and/or anteroposterior compression, which is further evidence that these patients were vocally hyperfunctional. An alternative explanation is that there were smoking- and/or surgery-related changes in the mechanical properties of the vocal fold tissues (eg, increased stiffness of the vocal fold cover) that

created the need for higher subglottal pressures to drive the vocal folds into vibration. The fact that normal mucosal wave activity was observed on most postsurgical vocal folds argues somewhat against this second interpretation.

It is not possible to determine from the present study if abnormally high subglottal pressures preceded and/or contributed to the formation of Reinke's edema, or if pressures became elevated after the formation of the edema as part of a reaction to the vocal difficulties that are associated with the presence of lesions on the vocal folds. However, the presence of abnormally high subglottal pressures in postsurgical patients is of concern, since this may reflect a pattern of vocal hyperfunction that could place such patients at increased risk for trauma-induced recurrence of polypoid lesions. A clear next step in the investigation of this disorder is to determine if postsurgical voice therapy can succeed in reducing the pressure required by these patients to comfortably phonate. Patient cohorts of smokers and nonsmokers would require analysis to determine if a reduction in subglottal pressure is related to a lower incidence of recurrence (especially in those who continue to smoke).

Of special interest was the patient with long-term vocal fold paralysis and severe reflux. This patient developed Reinke's edema despite not having a smoking history. It is our belief that the extremely high subglottal aerodynamic driving pressures that she required to achieve entrained glottal oscillation contributed to the polypoid changes in her mobile vocal fold. After phonomicrosurgical reduction of the polypoid changes of the mobile fold and subsequent medialization of the paralyzed fold 1 month later, the patient had a dramatic voice improvement.

The infrequency with which Reinke's edema presents with a paralyzed vocal fold may have a number of reasons. The paralyzed fold would need to be widely abducted to precipitate severe incompetence and possibly remain so for years. In turn, the individual would have to remain a vocal overuser despite the glottal incapacitation. Finally, there would probably need to be significant reflux mucositis of the larynx. Most likely, a constellation of these cofactors precipitated this unusual presentation of Reinke's edema in a nonsmoker that was not hypothyroid.

PHONOMICROSURGICAL CONSIDERATIONS

We believe that the superficial lamina propria is the primary layer site of pathologic change. Although some authors have noted that Reinke's edema can be frequently accompanied by keratosis with atypia, this was an unusual finding in this series. Therefore, the

suctioning technique was typically used, which maximally preserves the glottal epithelium and contours the superficial lamina propria to a more appropriate volume. Great care was taken to not remove too much volume of the superficial lamina propria in order to avoid adherence of the epithelial basement membrane to the vocal ligament. This type of adherence generally results in a stiffer oscillatory glottal system and impaired laryngeal sound production. Iatrogenic scarring and stiffness of the glottal epithelium would have a debilitating effect in this population, which uses significantly increased subglottal pressures to drive the previously volume-loaded glottal system. Adherence of the glottal epithelium to the medial lamina propria and generalized laryngeal hyperfunction explain the prolonged postoperative dysphonia that has been described in some patients.²⁹

Diffuse removal of the superior vocal fold (epithelium and superficial lamina propria) by means of stripping, cold microdissection, or laser vaporization can be effective treatment. However, we agree with Lumpkin et al¹⁵ that patients heal faster with the superior microflap and suctioning technique. Furthermore, precise contouring of the superficial lamina propria is relatively easy after raising the epithelial microflap (Fig 1D) and redraping it as necessary. In this series, with this technique, there was no significant epithelial scarring as judged by endoscopy with stroboscopy.

Controlling the reflux-induced laryngeal mucositis probably helps avoid recurrence of the Reinke's edema. On the basis of clinical observations during the postoperative period, we also believe that controlling laryngopharyngeal reflux is important for avoiding postsurgical scarring^{7,30} in the superficial lamina propria.

It is remarkable that the technique of vocal fold "stripping" was in fact designed by Lore³¹ in 1934 to be performed as a one-handed procedure through a monocular laryngoscope without magnification or general anesthesia. In fact, the majority of cases for which Lore³¹ and Putney and Clerf¹¹ initially used the stripping technique were Reinke's edema. The expanded superficial lamina propria provided a safety net for the poorly controlled tearing of the vocal fold cover. This, in turn, allowed for an improved postoperative voice despite the imprecision of the stripping technique.

PATHOPHYSIOLOGIC MECHANISMS

Yonekawa¹⁴ cited Toriya et al,³² who have proposed that Reinke's edema develops as a result of the combination of smoking and vocal abuse. This concept has been alluded to by a number of other authors.^{9-11,33-36} Yonekawa¹⁴ analyzed objective pre-

surgical and postsurgical acoustic measures and highlighted a number of observations, but did not offer explanations about the mechanism of development of this disorder. He, like others, acknowledged that the difficulty in developing theories about the origin of Reinke's edema is that patients present with the disorder already developed, and that no one has been studied in a prelesional state. It is unlikely that a cohort of patients can be identified and measured in a longitudinal fashion who have risk factors for Reinke's edema and then go on to develop it. Therefore, indirect means such as clinical observation, as well as objective vocal measures before and after surgery with extended follow-up and the inclusion of postsurgical voice therapy protocols, need to be used to shed further light on the etiologic mechanisms of Reinke's edema.

We agree with other investigators that most patients with Reinke's edema are vocal abusers and overusers, and that occasionally individuals may suffer from hypothyroidism. Typically, these patients demonstrate increased subglottic aerodynamic driving pressures. Individuals who exhibit this type of vocal hyperfunction, in an environment of chronic mucosal irritation from smoking and/or reflux with throat clearing and/or coughing, are predisposed to developing Reinke's edema. The increased aerodynamic driving pressure from the subglottic larynx results in unopposed distention of the superficial lamina propria and overlying epithelium on the superior surface of the vocal fold. This idea is in agreement with the findings of Kambic et al,²⁸ who examined the anatomic pathologic characteristics of Reinke's edema. The location of this distention is easily observed during stroboscopic and microlaryngoscopic examination.

Similar to what has been hypothesized for other types of hyperfunction-related vocal fold lesions,²⁷ Reinke's edema may involve a type of vicious circle in which the presence of anatomic alteration of the vocal folds (eg, inflammatory mucositis) makes voice production more difficult, requiring even higher levels of aerodynamic driving forces and myoelastic restoring forces. Such increases in the forces underlying the generation of phonation may then contribute to further mechanical trauma to the vocal folds and development or growth of lesions.

The superficial lamina propria does not typically distend as dramatically on the medial vocal fold surface, because Reinke's space is limited medially by the vocal ligament. The phonation pressure gradient below and above the glottis probably leads to permeability of small vascular and lymphatic channels in the superficial lamina propria. Conceptually, this bears similarity to the development of a serous otitis media. Histopathologic analysis of the superficial lamina propria with Reinke's edema reveals a myxoid, relatively acellular stroma. Immunocytochemical studies by Gray et al³⁷ have further characterized this extracellular matrix as having few structural proteins such as collagen and elastin, as well as little or no fibronectin. This finding explains the gelatinous deformability of the superficial lamina propria in Reinke's edema. During inspiration, due to the Bernoulli effect, the edema can sometimes appear to be medial. However, it has simply been displaced caudally due to mild inspiratory aerodynamic pressure.

Further investigations into the ultrastructural morphology of the matrix proteins in the superficial lamina propria of patients with Reinke's edema should elucidate the variable patterns of glottal oscillation that were observed during preoperative stroboscopy. At present, we are designing an enhanced stroboscopic grading system for analyzing patients with extensive polypoid corditis that will accommodate a variety of unique characteristics of this disorder.

SUMMARY

Endoscopic observation of Reinke's edema reveals extensive swelling of the superficial lamina propria on the superior surface of the vocal folds and abnormal supraglottic compression. This disorder is associated with an abnormally low average speaking F0 and increased subglottal driving pressures. These data support the hypothesis that Reinke's edema arises, in part, from long-standing vocal overuse and abuse that occurs in the anatomic environment of smoking and/or possibly reflux. Observations on the postsurgical voice analyses suggest that these patients continue to hyperfunction and that management strategies that include vocal therapy as well as smoking cessation may be beneficial for preventing recurrence.

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VOCAL FOLD ATROPHY: QUANTITATIVE GLOTTIC MEASUREMENT AND VOCAL FUNCTION

KOICHI OMORI, MD

KOBE, JAPAN

HISAYOSHI KOJIMA, MD

KYOTO, JAPAN

DAVID H. SLAVIT, MD

NEW YORK, NEW YORK

ASHUTOSH KACKER, MD

NEW YORK, NEW YORK

CARLOS MATOS, MD

PORTO, PORTUGAL

STANLEY M. BLAUGRUND, MD

NEW YORK, NEW YORK

Videostroboscopic glottic measurements and vocal function were evaluated in 41 vocal fold atrophy patients with bowed vocal folds. The amount of bowing in the resting position and the glottal gap area and vibratory amplitude during phonation were measured from digitized videostroboscopic images. Vibratory amplitude was not decreased on atrophic vocal folds. With the same amount of total bowing, the glottal gap area for bilateral atrophy was smaller than for unilateral atrophy. These results suggest that vocal fold atrophy is not disadvantageous to thyroplasty type I, and that bilateral procedures may produce a better outcome than a unilateral procedure in the treatment of bilateral atrophy. Acoustic, aerodynamic, and perceptual parameters of vocal function were measured. The acoustic high-frequency power ratio and the H-index correlated with the glottal gap area. The mean flow rate correlated with the amount of bowing. The degree of dysphonia was related to the size of the glottal gap and bowing.

KEY WORDS — glottic measurement, image analysis, videostroboscopy, vocal fold atrophy, vocal function.

INTRODUCTION

Vocal fold atrophy involves either the muscle, the mucosa, or another structure in the vocal fold.¹ The deformity results in a glottal gap, producing a breathy voice and inefficient phonation. Lindestad and Hertegård² analyzed vocal function of vocal fold atrophy patients with acoustic, aerodynamic, and perceptual measures. With the further development of videostroboscopic techniques, objective glottic measurements based on video analysis have been introduced.^{3,4} How does the atrophic vocal fold vibrate compared to normal? What are the implications regarding thyroplasty type I? The purpose of this study is to ascertain physiological characteristics of vocal fold atrophy by the combination of glottic measurement and vocal function study. The degree of vocal fold atrophy was quantified on the digitized laryngeal image, and the glottal gap and vibratory amplitude were measured. Acoustic, aerodynamic, and perceptual parameters were measured. The relationships between glottic measures and vocal function measures were analyzed.

SUBJECTS

All patients had videolaryngoscopic findings of at least one bowed vocal fold with or without sulcus vocalis. Those patients who had laryngeal paralysis, laryngeal trauma, previous laryngeal surgery, laryngeal elevated lesions (polyp, nodule, neoplasm, etc), or neurologic disorders that could alter vocal func-

tion were excluded from the present series. Forty-one patients, 16 men and 25 women, were studied. Vocal fold atrophy was unilateral in 17 patients (6 men and 11 women) and bilateral in 24 patients (10 men and 14 women). Eight patients had sulcus vocalis according to videolaryngoscopic observation (6 unilateral and 2 bilateral sulcus). Patient ages ranged from 25 to 87 years. The group demographics are shown in Table 1.

METHODS

Glottic Measurement. Glottic measurements from videostroboscopic image analysis were performed with previously described techniques.⁴ Briefly, vocal fold vibration during phonation of a sustained vowel /i/ at the patient's habitual pitch and loudness was monitored and recorded through a 70° telescope (Nagashima Co, Tokyo, Japan) connected to a charge-coupled device video camera (Toshiba Corp, Tokyo) with a stroboscopic light source (Nagashima Co). Laryngostroboscopic images were recorded with a CVD-1000 Hi8 computer video deck (Sony Corp, Tokyo) at 30 frames per second. Recorded sequential images of vocal fold vibration were played back frame by frame. Laryngeal images were digitized with a Color Space II and Color Space IIfx video-graphics card (MASS Microsystems Inc, Sunnyvale, Calif) and stored in a Macintosh IIfx computer (Apple Computer Inc, Cupertino, Calif).

Laryngeal images were retrieved by Image soft-

From the Ames Vocal Dynamics Laboratory, Lenox Hill Hospital, New York, New York (Omori, Slavitt, Matos, Kacker, Blaugrund), and the Department of Hearing and Speech Science, Kyoto University Graduate School of Medicine, Kyoto, Japan (Kojima).

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CORRESPONDENCE — Koichi Omori, MD, Dept of Otolaryngology, Nishi-Kobe Medical Center, Kouji-dai 5-7-1, Nishi-ku, Kobe 651-22, Japan.

TABLE 1. SUBJECT DEMOGRAPHICS

Case No.	Sex	Age (y)	Bowing	Sulcus
1	M	27	B	
2	M	43	B	
3	M	53	B	
4	M	66	B	R
5	M	71	L	
6	M	71	B	
7	M	76	B	R
8	M	76	B	
9	M	83	L	
10	F	25	B	
11	F	28	B	
12	F	32	R	
13	F	32	L	
14	F	36	B	
15	F	45	L	
16	F	49	B	B
17	F	51	B	R
18	F	52	L	
19	F	55	B	
20	F	61	B	
21	F	72	L	
22	F	83	B	B
23	M	30	B	R
24	M	65	L	
25	F	26	B	
26	F	35	L	L
27	F	35	B	
28	F	39	B	
29	F	55	L	
30	F	60	B	
31	M	47	L	
32	M	65	R	R
33	M	67	B	
34	M	87	R	
35	M	87	B	
36	F	54	R	
37	F	60	L	
38	F	68	L	
39	F	73	B	
40	F	74	B	
41	F	77	L	

B — bilateral, R — right, L — left.

ware (National Institutes of Health, Bethesda, Md) and displayed on a monitor. At the resting position of the vocal folds, the amount of bowing was quantified as shown in Fig 1. The maximum distance (d) between the edge of the vocal fold and the line connecting the anterior commissure and the tip of the vocal process was measured. The distance (d) was normalized by the membranous vocal fold length (L) and termed the bowing index (BI). The formula is $BI = d/L \times 100$ (units). The total BI was calculated by adding

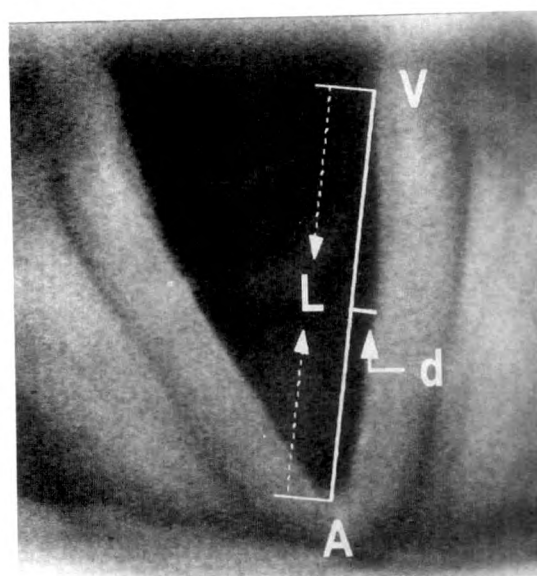


Fig 1. Measurement of amount of bowing. A — anterior commissure, d — distance from line connecting anterior commissure and tip of vocal process to edge of vocal fold, L — vocal fold length, V — tip of vocal process.

the sum of the right and left BIs.

From the sequential stroboscopic images, the glottal gap area at the maximum closed point of a vibratory cycle and the amplitude of vocal fold vibration during the vibratory cycle were measured as shown in Figs 2 and 3. The glottal gap area was normalized by the square of the membranous vocal fold length, and termed the normalized glottal gap area (NGGA). The formula is $NGGA = \text{glottal gap area}/L^2 \times 100$ (units). The amplitude was determined by the difference between the vocal fold width at the maximum closed point and that at the maximum opened point of vibration. The amplitude was normalized by the membranous vocal fold length, and termed the normalized amplitude (NA). The formula is $NA = \text{amplitude} (a1 - a2)/L \times 100$ (units). The total NA was calculated by adding the sum of the right and left NAs.

Vocal Function Study. The acoustic analysis included pitch perturbation (jitter), amplitude perturbation (shimmer), harmonics-to-noise ratio (HN),⁵ and high-frequency power ratio (HPR).⁶ A sustained vowel sound /a/ in comfortable phonation at habitual pitch and loudness was examined. The acoustic analysis also included the H-index, determined from phonation of the consonant-vowel combination /hai/.⁷

A B&K 4006 (Naerum, Denmark) condenser microphone and a Sony PCM-2500A digital audiotape recorder were used for data acquisition. Sound collection was performed in a sound-treated booth. The distance from the microphone was set at 20 cm. The data, stored on digital audiotape, were then transferred to a Macintosh IIX computer through a DAT

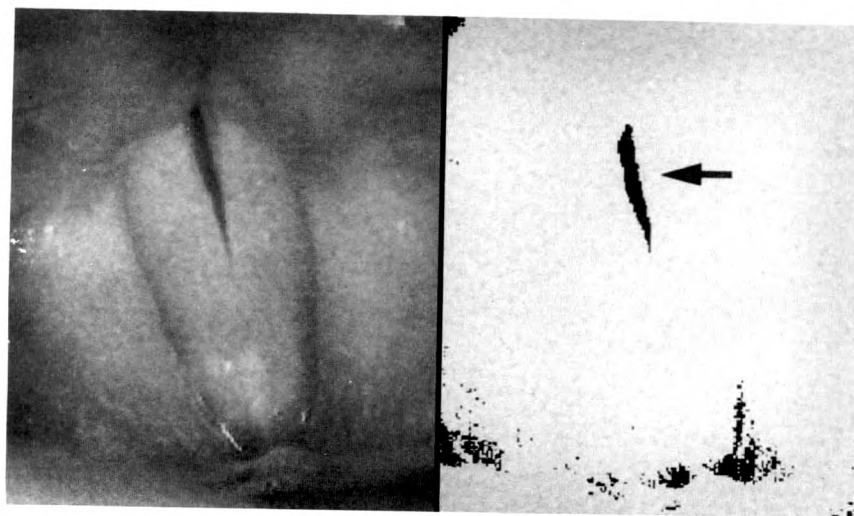


Fig 2. Measurement of glottal gap area (arrow).

input-output direct digital interface (Digidesign Inc, Menlo Park, Calif). All analyses were performed with a software (C language) program written by the members of the Ames Vocal Dynamics Laboratory.^{6,7}

The aerodynamic analysis included mean flow rate (MFR), AC/DC ratio, and maximum phonation time (MPT). These parameters were calculated from a sustained vowel sound /u/ in comfortable phonation. Measurements of airflow were made with a PA 500 Phonation Analyzer high-frequency response hot-wire flowmeter (Minato Co, Tokyo).

Perceptual evaluations of the degree of breathiness of the sustained vowel sound /a/ were determined by playing the digitized sound file of a voice sample through a 16-bit digital-to-analog converter and Bose speakers (Boston, Mass). The degree of breathiness was scored 0, 1, 2, or 3 by three experienced otolaryngologists. A score of 3 was given for severely breathy voices, a score of 2 was given for moderately breathy voices, a score of 1 was given for slightly

breathy voices, and a score of 0 was given for normal voices. The scores judged by three listeners were averaged and classified again into four categories: scores of 0, 1, 2, or 3.

The normative ranges for these measures were determined from the evaluation of 100 normal volunteers (50 men, 50 women) without laryngeal diseases at the Ames Vocal Dynamics Laboratory, Lenox Hill Hospital.

All statistical analyses in this study were performed with SPSS software (SPSS Inc, Chicago, Ill).

RESULTS

Glottic Measurement. Glottic measurements were undertaken on the digitized laryngeal images in 30 of 41 patients (cases 1 through 30): 10 unilateral and 20 bilateral vocal fold atrophy patients, and 23 nonsulcus patients and 7 sulcus patients. The results of glottic measurements are summarized in Table 2. The total BI ranged from 1.6 to 6.5 units in the unilateral

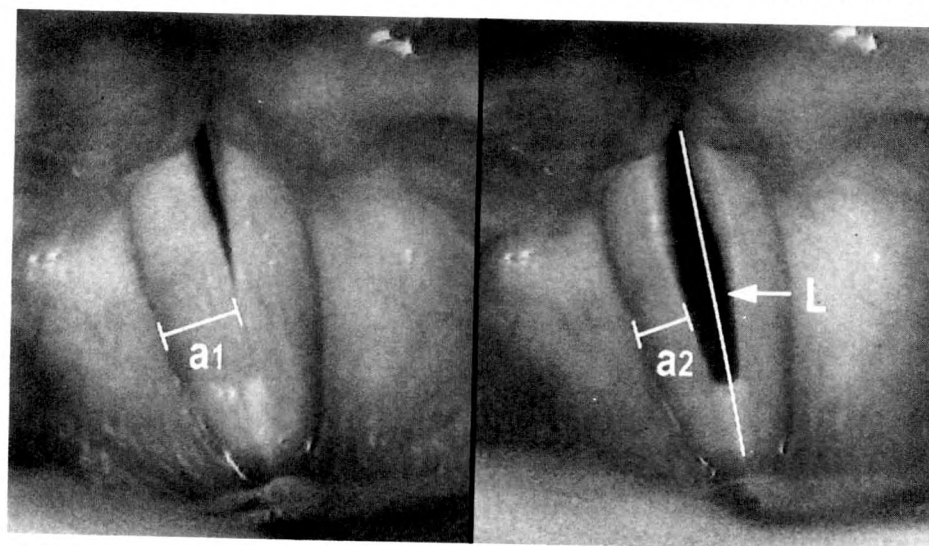


Fig 3. Measurement of vibratory amplitude. L — vocal fold length, a1 — vocal fold width at maximum closed point of vibration, a2 — vocal fold width at maximum opened point of vibration.

TABLE 2. RESULTS OF GLOTTIC MEASUREMENTS

	Laterality		Existence of Sulcus	
	Unilateral	Bilateral	Nonsulcus	Sulcus
Total BI	3.9 ± 1.5	6.7 ± 2.3	5.5 ± 2.5	6.8 ± 1.8
NGGA	0.9 ± 1.0	0.8 ± 0.9	0.7 ± 0.9	1.2 ± 1.1
Total NA	13.1 ± 3.9	11.7 ± 5.1	11.4 ± 4.8	14.8 ± 3.4

Data are mean ± SD in units.
BI — bowing index, NGGA — normalized glottal gap area, NA — normalized amplitude.

atrophy group (mean ± SD, 3.9 ± 1.5) and ranged from 2.5 to 11.4 units in the bilateral atrophy group (mean 6.7 ± 2.3). There was a significant difference in total BI between the unilateral and bilateral atrophy groups by analysis of variance (ANOVA). The total BI ranged from 1.6 to 11.4 units in the nonsulcus group (mean 5.5 ± 2.5) and ranged from 4.6 to 9.3 units in the sulcus group (mean 6.8 ± 1.8). There was no significant difference in total BI between the nonsulcus and sulcus groups by ANOVA.

The NGGA ranged from 0 to 3.1 units in the unilateral atrophy group (mean 0.9 ± 1.0) and from 0 to 3.3 units (mean 0.8 ± 0.9) in the bilateral atrophy group. There was no significant difference in NGGA between unilateral and bilateral atrophy groups by ANOVA. Complete glottic closure was observed in 1 of 10 unilateral atrophy cases (10%) and in 9 of 20 bilateral atrophy cases (45%). The NGGA ranged from 0 to 3.3 units in the nonsulcus group (mean 0.7 ± 0.9) and from 0 to 3.1 units (mean 1.2 ± 1.1) in the sulcus group (Table 2). There was no significant difference in NGGA between the nonsulcus and sulcus groups by ANOVA. Complete glottic closure was observed in 8 of 23 nonsulcus cases (35%) and in 2 of 7 sulcus cases (29%).

With regard to the amplitude of vocal fold vibration, the total NA ranged from 8.8 to 20.6 units in the

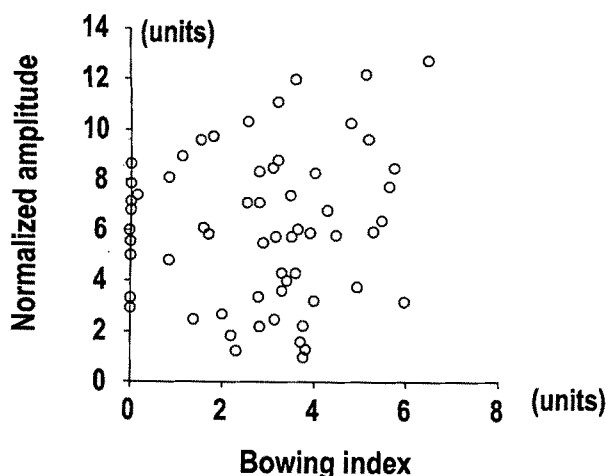


Fig 4. Relationship between bowing and amplitude.

TABLE 3. NORMALIZED AMPLITUDE IN UNILATERAL VOCAL FOLD ATROPHY GROUP

Case No.	Right (units)	Left (units)
5	7.1	8.3*
9	6.8	3.2*
12	5.9*	2.9
13	5.6	8.7*
15	8.6	5.8*
18	7.8	12.8*
21	5.0	3.8*
24	3.0	7.4*
26	5.8	5.8*
29	7.4	9.6*

*Atrophic side.

unilateral atrophy group (mean 13.1 ± 3.9) and from 3.7 to 21.8 units (mean 11.7 ± 5.1) in the bilateral atrophy group. There was no significant difference in total NA between the unilateral and bilateral atrophy groups by ANOVA. The total NA ranged from 3.7 to 21.4 units in the nonsulcus group (mean 11.4 ± 4.8) and from 11.5 to 21.8 units (mean 14.8 ± 3.4) in the sulcus group. By ANOVA, there was no significant difference between the nonsulcus and sulcus groups for total NA.

The data were analyzed to determine if a relationship exists between the degree of bowing and the vibratory amplitude on the individual vocal fold. The BI ranged from 0 to 6.5 units, and the individual vocal fold NA ranged from 1.0 to 12.2 units. Figure 4 illustrates the finding of no significant relationship between the BI and NA (Pearson's correlation coefficients). In the 10 unilateral vocal fold atrophy cases, there was no significant difference in NA between the atrophic side and the nonatrophic side by ANOVA (Table 3).

The relationship between the amount of total bowing and glottal gap area is shown in Fig 5. With the

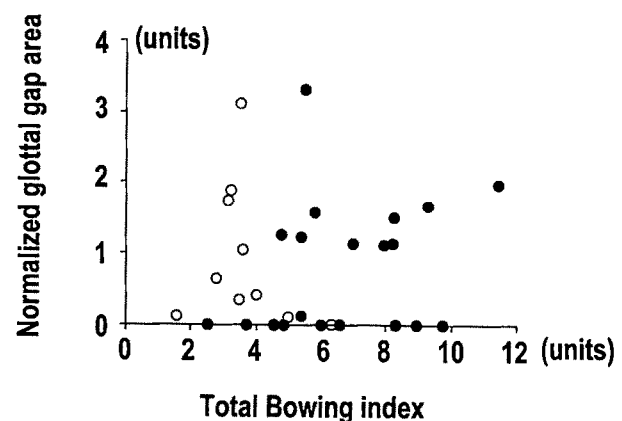


Fig 5. Relationship between bowing and glottal gap. Open circle — unilateral atrophy, closed circle — bilateral atrophy.

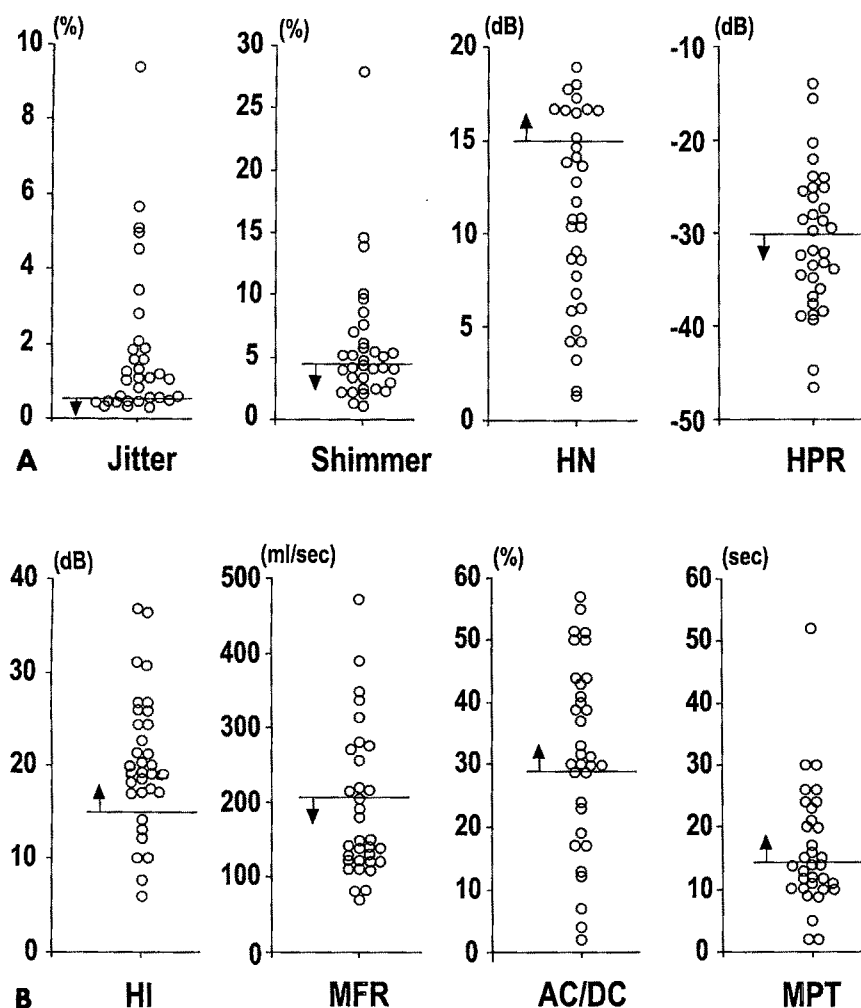


Fig 6. Acoustic and aerodynamic measures. Arrows indicate normal ranges. A) Results of jitter, shimmer, harmonics-to-noise ratio (HN), and high-frequency power ratio (HPR). B) Results of H-index (HI), mean flow rate (MFR), AC/DC ratio, and maximum phonation time (MPT).

same amount of total bowing (total BI between 0 and 6.5 units), the glottal gap area was smaller for bilateral atrophy cases than for unilateral atrophy cases, although there was no significant difference in glottal

TABLE 4. RESULTS OF ACOUSTIC AND AERODYNAMIC MEASURES

	Laterality		Existence of Sulcus	
	Unilateral	Bilateral	Nonsulcus	Sulcus
Jitter (%)	1.5 ± 1.5	2.0 ± 2.4	1.9 ± 2.2	1.3 ± 1.3
Shimmer (%)	6.3 ± 6.8	5.5 ± 3.6	6.1 ± 5.5	4.6 ± 2.9
HN (dB)	11.7 ± 5.0	10.6 ± 5.5	11.4 ± 5.4	9.8 ± 4.7
HPR (dB)	-31.8 ± 8.0	-30.1 ± 7.3	-30.4 ± 7.5	-32.5 ± 8.0
H-index (dB)	20.8 ± 6.8	20.1 ± 8.3	19.7 ± 7.6	24.0 ± 7.7
MFR (mL/s)	190 ± 111	192 ± 90	194 ± 101	180 ± 91
AC/DC (%)	30 ± 17	33 ± 14	31 ± 16	38 ± 10
MPT (s)	16 ± 8	17 ± 11	17 ± 10	14 ± 4

Data are mean ± SD.

HN — harmonics-to-noise ratio, HPR — high-frequency power ratio, MFR — mean flow rate, MPT — maximum phonation time.

gap area on the average between the two groups.

Vocal Function Study. Vocal function was evaluated by acoustic, aerodynamic, and perceptual measures in 33 of 41 patients (cases 1 through 22, and cases 31 through 41). Figure 6 shows the acoustic and aerodynamic measures. Jitter ranged from 0.3% to 9.4%, with 20 cases outside the normal range (61% of subjects). Shimmer ranged from 1.1% to 27.9%, with 16 cases outside the normal range (48% of subjects). The HN ranged from 1.3 to 18.9 dB, with 23 cases outside the normal range (70% of subjects). The HPR ranged from -14.0 to -44.7 dB, with 16 cases outside the normal range (48% of subjects). The H-index ranged from 6.0 to 36.8 dB, with 7 cases outside the normal range (21% of subjects). The MFR ranged from 70 to 472 mL/s, with 12 cases outside the normal range (36% of subjects). The AC/DC ratio ranged from 2% to 57%, with 12 cases outside the normal range (36% of subjects). The MPT ranged from 2 to 52 seconds, with 18 cases outside the normal range (55% of subjects).

In the 33 cases, there were 14 unilateral and 19 bilateral atrophy cases, and 27 nonsulcus and 6 sulcus

TABLE 5. PERCEPTUAL SCORES OF BREATHINESS BY LATERALITY AND EXISTENCE OF SULCUS

Perceptual Score*	Laterality		Existence of Sulcus	
	Unilateral	Bilateral	Nonsulcus	Sulcus
0	6	7	10	3
1	7	9	13	3
2	0	3	3	0
3	1	0	1	0

Data are numbers of subjects.

*0 — normal, 1 — slightly breathy, 2 — moderately breathy, 3 — severely breathy.

cases. The results of acoustic and aerodynamic measures are summarized in Table 4. There was no significant difference between the unilateral and bilateral atrophy groups for all these measures. There was also no significant difference between the nonsulcus and sulcus groups for all these measures. Table 5 shows the results for perceptual scores of breathiness. In 20 of 33 cases (61%), the voice was perceived as breathy.

Relationships Between Glottic Measures and Vocal Function Measures. Relationships between vocal function measures (jitter, shimmer, HN, HPR, H-index, MFR, AC/DC ratio, and MPT) and quantitative glottic measures (total BI, NGGA, and total NA) were analyzed by use of the data from the 22 of 41 patients (cases 1 through 22) who underwent both groups of analysis. Table 6 summarizes the relationships between glottic measures and vocal function measures. The HPR and H-index had significant correlations with the NGGA ($p < .05$), while the MFR had a significant correlation with the total BI ($p < .05$) by the analysis of Pearson's correlation coefficients. Figure 7 shows the relationship between NGGA and HPR. Figure 8 illustrates the relationship between total BI and MFR.

DISCUSSION

Vocal fold atrophy is usually diagnosed by the laryngoscopic finding of a bowed vocal fold or a thin vocal fold.¹ The present study included only vocal fold atrophy patients with bowed vocal folds. "Bowed"

TABLE 6. RELATIONSHIPS BETWEEN GLOTTIC MEASURES AND VOCAL FUNCTION MEASURES

	Total BI	NGGA	Total NA
Jitter (%)	NS	NS	NS
Shimmer (%)	NS	NS	NS
HN (dB)	NS	NS	NS
HPR (dB)	NS	$p < .05$	NS
H-index (dB)	NS	$p < .05$	NS
MFR (mL/s)	$p < .05$	NS	NS
AC/DC	NS	NS	NS
MPT (s)	NS	NS	NS

NS — not significant. (See Tables 2 and 4 for other abbreviations.)

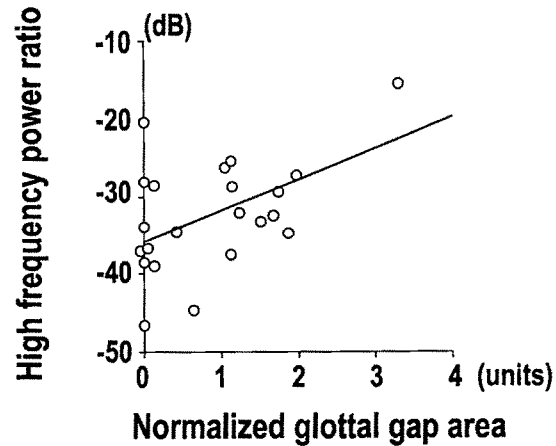


Fig 7. Relationship between glottal gap area and high-frequency power ratio.

designates a concave edge of the vocal fold, unrelated to whether the vocal fold mass appears small, normal-sized, or large. Those patients who had thin vocal folds without bowing were excluded from this series, because there is no objective evaluation for abnormality of vocal fold width.

Isshiki et al⁸ classified vocal fold atrophy into five etiologic groups: vocal abuse, aging, trauma (including prior laryngeal operation), psychologic causes, and mutation. Tanaka et al⁹ presented 12 patients who had bowing of the vocal fold without any noticeable organic lesion. Most of the patients in their study were male and older than 60 years. They could not determine the cause of bowing in young adults. Our present study excluded patients who had laryngeal paralysis, laryngeal trauma, previous laryngeal surgery, laryngeal elevated lesions (polyp, nodule, neoplasm, etc), and neurologic disorders. Because electromyographic examination was not undertaken in our study, cases with reduced activity of laryngeal muscles may or may not be included. However, all the cases had normal vocal fold movements of abduction and adduction in the present study.

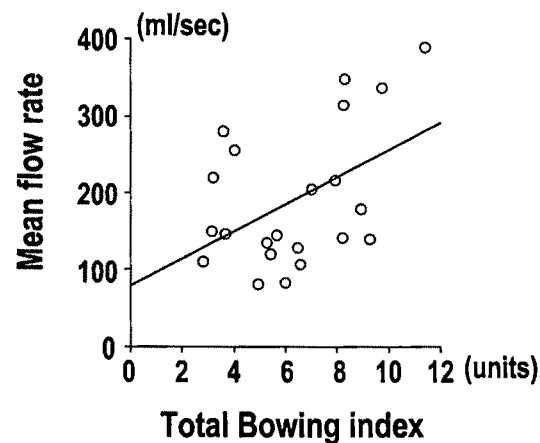


Fig 8. Relationship between bowing and mean flow rate.

This paper presents static and dynamic glottic measurements of vocal fold atrophy utilizing digitized laryngostroboscopic images. The BI allowed quantitative measurement and documentation of the amount of vocal fold bowing. Glottal gap area and vibratory amplitude were also objectively measured with a previously reported technique.⁴

Lindestad and Hertegård² reported that 57% (29 out of 51 cases) of patients with spindle-shaped glottal insufficiency without sulcus vocalis had incomplete glottic closure according to a visual-perceptual stroboscopic evaluation. This contrasts with our finding, based on quantitative glottic measurements, of incomplete glottic closure in 65% of vocal fold atrophy patients without sulcus vocalis. This difference may be partly related to our precise quantitative investigation of glottal gap on the stroboscopic sequential images.

With regard to the amplitude of vocal fold vibration, on our quantitative glottic measurement it was noted that vibratory amplitude was not significantly decreased in the presence of atrophic vocal folds. This may be because the amplitude of vocal fold vibration is dependent on the glottal gap and the stiffness of the vocal folds. The atrophic vocal folds did not have a decreased amplitude of vibration nor stiffened mucosa. These results support the belief that vocal fold atrophy is not disadvantageous to framework surgery such as thyroplasty type I. Isshiki et al⁸ reported the effectiveness of thyroplasty type I for vocal fold atrophy cases.

From our glottic measurement, complete glottic closure was observed in bilateral atrophy cases more often than in unilateral atrophy cases. With the same amount of total bowing, the glottal gap area for bilateral atrophy was smaller than that for unilateral atrophy. Symmetric vocal folds were better for glottic closure of vocal fold vibration than were asymmetric vocal folds. In the treatment of bilateral vocal fold atrophy, bilateral thyroplasty type I may produce a better outcome than a unilateral procedure.

For the various acoustic and aerodynamic measurements, 30% to 79% of vocal fold atrophy patients had values within the normal range. Furthermore, perceptual scores showed that 39% of vocal fold atrophy patients had a normal (nonbreathy) voice quality. These results suggest that acoustic, aerodynamic, and perceptual parameters cannot separate subjects with vocal fold atrophy from normal subjects.

There were no significant differences in vocal function between the group of patients with unilateral

atrophy and the group with bilateral atrophy. Between the nonsulcus and sulcus groups, there were no significant differences in vocal function. Similarly, Lindestad and Hertegård² showed no differences in voice characteristics between nonsulcus and sulcus groups of patients with spindle-shaped glottal insufficiency. Ford et al¹⁰ classified sulcus vocalis into three types based on histopathologic analysis. Our diagnosis of sulcus vocalis was based on laryngoscopic findings of sulcus in the vocal fold. Various types of sulcus vocalis may be included in the present study.

Combining the acoustic and aerodynamic measurements with the objective glottic measurements revealed numerous statistically significant relationships. The HPR and H-index had significant correlations with NGGA. The aerodynamic measure, MFR, had a significant correlation with the total BI. The degree of dysphonia was related more to the amount of bowing and the size of the glottal gap than to the existence of sulcus vocalis or the presence of unilateral versus bilateral vocal fold atrophy.

The present technique of videostroboscopic image analysis was limited by the nature of both stroboscopy and telescopic. In this study, all patients who had only flexible fiber-optic endoscopy were excluded because of optical distortion with this technique. Yanagisawa and Yanagisawa¹¹ demonstrated that a telescopic view had no distortion when the telescope was positioned in the midline of the tongue and oropharynx. Objective video analysis can only be performed accurately when the image displays the anterior commissure and the tip of the vocal process simultaneously. Stroboscopy is not suited to the assessment of patients with severe aperiodic vibration of the vocal fold.

Another limitation of quantitative video analysis is the lack of absolute numbers. Relative units of degree of bowing, glottal gap area, and amplitude were used, because exact measurements cannot be obtained with the present technology. The normalization is limited by the fact that vocal fold length is affected by pitch, loudness, and compensatory activity of extrinsic muscles. In an effort to control for these variables, the glottal gap area and amplitude were measured at the patient's habitual pitch and loudness in this study.

These glottic measurements will be valuable for quantification of the effects of vocal fold atrophy. Various methods, including voice training, intracordal injection, and thyroplasty type I, have been recently attempted for the treatment of dysphonia in vocal fold atrophy patients.^{8,10,12} The present study demonstrates the use of laryngeal image analysis to document the amount of bowing, the size of the glottal gap, and the

amplitude of vocal fold vibration in patients with vocal fold atrophy. These glottic measures, as well as acoustic and aerodynamic measures of vocal function, can help evaluate treatments of vocal fold atrophy.

SUMMARY

1. Vocal fold atrophy was investigated by the combination of glottic measurements and vocal function study.
2. The vibratory amplitude was not decreased on

atrophic vocal folds. With the same amount of total bowing, the glottal gap area for bilateral atrophy was smaller than for unilateral atrophy.

3. Acoustic, aerodynamic, and perceptual parameters cannot separate subjects with vocal fold atrophy from normal subjects.

4. The acoustic HPR and H-index correlated with glottal gap area. The MFR correlated with the amount of total bowing. The degree of dysphonia was related to the size of the glottal gap and bowing.

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CHANGES IN LENGTH AND SPATIAL ORIENTATION OF THE VOCAL FOLD WITH ARYTENOID ADDUCTION IN CADAVER LARYNGES

GAYLE E. WOODSON, MD

MEMPHIS, TENNESSEE

ARNE HENGESTEG

MEMPHIS, TENNESSEE

DANIEL YEUNG, PHD

MEMPHIS, TENNESSEE

CLARK A. ROSEN, MD

PITTSBURGH, PENNSYLVANIA

NAIDY CHEN, MS

MEMPHIS, TENNESSEE

Videendoscopy suggests that arytenoid adduction (AA) surgery not only medializes the paralyzed vocal fold, but increases the length of its membranous portion so that it more closely resembles the normal side. This could represent either real length change or out-of-plane rotation. Computed tomography scanning was performed on adult male cadaver larynges before and after the AA procedure to measure changes in length and spatial orientation of the vocal fold. Three-dimensional coordinates of radiopaque markers on the anterior commissure, posterior glottic midline, and vocal processes were determined. The distance between the vocal processes was 3.9 mm before, and 0.8 mm after AA. The mean vocal fold length was 12.4 mm before, and 13.4 mm after AA ($p = .14$). The vocal process moved consistently caudally, an average of 3.5 mm ($p = .02$). The data suggest that clinically apparent vocal fold length changes with AA could be an illusion due to vertical displacement of the vocal process, and not actual lengthening.

KEY WORDS — larynx, paralysis, treatment results.

INTRODUCTION

The development of office videolaryngoscopy has considerably advanced our knowledge of glottal function in phonation, as well as our understanding of impaired function in patients with unilateral laryngeal paralysis. The symptoms of laryngeal paralysis are related not only to the size of the glottal gap, but also to changes in the spatial orientation and muscle bulk of the vocal fold. Brewer et al¹ reviewed video recordings of patients with unilateral laryngeal paralysis and observed that the paralyzed vocal fold consistently appeared shorter than the normal vocal fold. Subsequently, our observations in both humans and experimental animals have confirmed that in the two-dimensional image, during inspiration the membranous portion of the paralyzed vocal fold appears considerably shorter than that of the normal side.^{2,3} The length difference between the two sides of the image decreases significantly during sustained phonation, due to compensatory shortening of the normal vocal fold. This compensatory activity involves hyperfunction, and it is difficult for the patient to achieve satisfactory glottic closure during connected speech.

Static surgical procedures for the treatment of unilateral laryngeal paralysis, including vocal fold

injection, type I thyroplasty, and the arytenoid adduction (AA) procedure, are intended to move the vibratory edge of the paralyzed vocal fold to the midline to permit apposition with the mobile fold during phonation and swallowing. It is generally accepted that vocal fold injection does not adequately close posterior laryngeal gaps.⁴ Type I thyroplasty does not directly manipulate the arytenoid cartilage, and its efficacy for posterior glottal gaps is controversial; however, some authors report successful medialization of the vocal process with this technique.⁵ The effect of either technique on vocal fold length has not been reported.

The AA procedure employs a suture attached to the muscular process to rotate the arytenoid, medializing the vocal process.⁶ The membranous vocal fold is dragged medially by this procedure, but incompetence of the anterior larynx due to muscle atrophy is not addressed. The AA procedure may be performed in combination with type I thyroplasty or vocal fold injection.

In a series of 13 laryngeal paralysis patients, analysis of videolaryngoscopy revealed that in the 10 patients with excellent vocal results after AA, the membranous vocal fold appeared to be lengthened so

From the Department of Otolaryngology, University of Tennessee, Memphis (Woodson, Hengesteg), and the Department of Radiology, St Jude Children's Research Hospital (Yeung, Chen), Memphis, Tennessee, and the Eye and Ear Institute, University of Pittsburgh, Pittsburgh, Pennsylvania (Rosen).

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CORRESPONDENCE — Gayle E. Woodson, MD, Dept of Otolaryngology, University of Tennessee, Memphis, 956 Court Ave, Memphis, TN 38163.

that vocal fold approximation could be achieved more easily, without compensatory hyperfunction.⁷ On the other hand, in the 3 patients with unsatisfactory results, the vocal process was adequately medialized, but vocal fold length was unchanged by surgery. In these patients, phonation still involved compensatory hyperfunction, with foreshortening of the glottis. These data suggest that to be successful, AA must lengthen the vocal fold. However, this conclusion is based on analysis of a two-dimensional image. Shortening of a structure in such an image could be merely an illusion caused by out-of-plane motion of the structure. Upward or downward motion of the vocal fold would result in rotation of the vocal fold, altering the length of its video image, even though there may be no actual change in the length of the vocal fold itself.

Does AA really increase the length of the vocal fold? Or does it merely displace the vocal process rostrally or caudally so that the two-dimensional image is longer? No matter which three-dimensional account of the motion is accurate, the spatial change reflected in the two-dimensional image appears to be crucial to the clinical efficacy of the procedure. We studied the spatial effects of AA in cadaver larynges using computed tomography (CT) scanning. We previously reported the use of a similar technique to measure rotation of the arytenoid.⁸ In the present study, markers were placed to permit calculation of spatial dimensions. We hypothesized that AA would medialize the vocal process and increase the length of the membranous vocal fold.

METHODS

Four freshly harvested cadaver larynges were used in this study, for a total of eight AA procedures. Radiopaque markers (1 × 1-mm particles of radiopaque polyethylene tubing) were sutured to the anterior commissure, vocal processes, and posterior-superior midline of the cricoid cartilage. Each specimen was suspended via six-point fixation in a saline-filled Plexiglas box for CT scanning with the larynx in the cadaveric position and after bilateral AA procedures.

In the AA procedure, mucosa was elevated from the pyriform sinus to expose the posterior cricoarytenoid muscle, whose fibers were traced to their insertion on the muscular process of the arytenoid. A curved needle was used to place a suture through the muscular process. Neither the cricothyroid joint nor the cricoarytenoid joint was disarticulated. All ligamentous attachments were preserved. Soft tissues were then dissected from the medial surface of the thyroid cartilage to create a tunnel from the muscular process to the area where the sutures would be se-

cured anteriorly. A straight needle was used to pass the two long ends of the sutures through the thyroid cartilage, 3 to 4 mm from the caudal edge, and 10 and 13 mm, respectively, from the anterior angle. Tension was adjusted to be just adequate to achieve maximal rotation of the arytenoid.

Axial scans were acquired with a Siemens Somatom DRH scanner, with contiguous slices at 2-mm thickness. The CT images were reconstructed with a 256 × 256 matrix with a pixel size of 0.3 mm.

Data from the CT scans were analyzed with three-dimensional imaging software. Multiple CT images were displayed. The images were zoomed and the contrast was adjusted to help identify the radiopaque markers. Each marker was labeled by defining a fiducial mark at its center. The axial plane was defined as the XY plane, with X increasing toward the right, and Y increasing from anterior to posterior. The Z axis was perpendicular to the axial plane, with increasing values from rostral to caudal. The Z coordinate of each fiducial mark was determined by its slice position, and the X and Y coordinates were determined by the cursor position within the slice, all relative to an arbitrarily chosen origin.

Medialization of the vocal processes was determined by measuring the distance from one vocal process to the other. The length of each membranous vocal fold was defined as the distance between the anterior commissure (ac) and the corresponding vocal process (vp). The distance between the two points was calculated by taking the square root of the sum of the squares of the differences between the coordinates in each plane of reference:

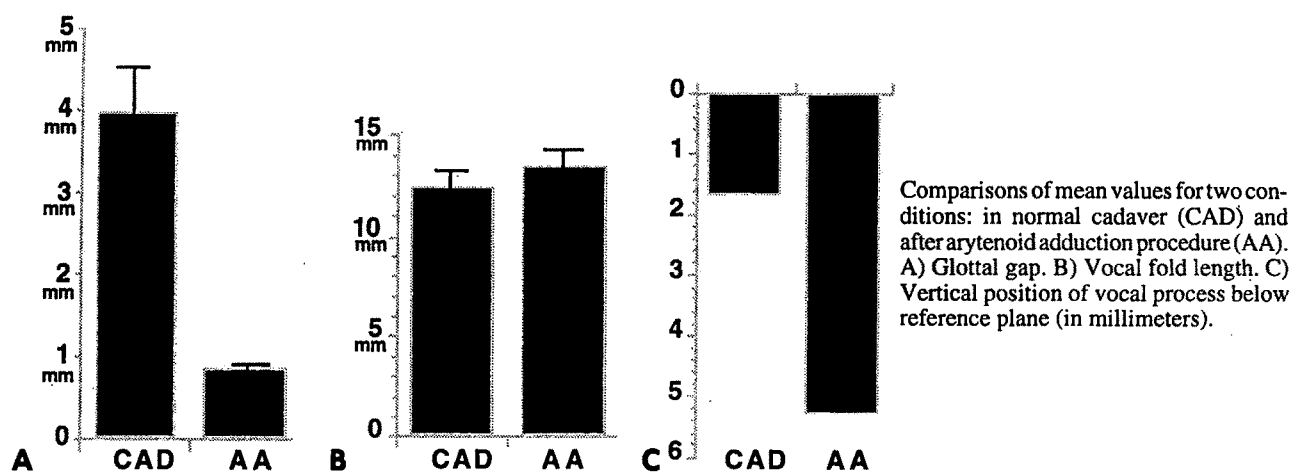
$$\text{Vocal fold length} = \sqrt{(X_{ac} - X_{vp})^2 + (Y_{ac} - Y_{vp})^2 + (Z_{ac} - Z_{vp})^2}$$

Changes in the rostrocaudal position of each vocal process were determined relative to a reference glottic plane passing through the anterior commissure and the posterior glottic midline and perpendicular to the sagittal plane. This method was chosen because simple measurement of the displacement in the Z axis did not control for subtle changes in the position of the larynx with respect to the scanner. The distance of each vocal process from the reference plane was determined in the Z axis before and after AA.

RESULTS

The Figure, A, displays the mean values for the glottal gap in the two conditions. The vocal processes were an average of 3.9 mm apart in the cadaveric position, decreasing to 0.8 mm after AA. This indicates significant vocal fold medialization achieved by AA.

The mean vocal fold length, as defined by the



distance from the anterior commissure to the vocal process, was 12.4 mm in the cadaveric position, and 13.4 mm after AA (see Figure, B). Hence, the length of the membranous vocal fold was not significantly altered by AA.

Each vocal process moved inferiorly with AA, an average distance of 3.5 mm (see Figure, C). The AA consistently produced caudal displacement of the vocal process, resulting in a caudal rotation of the membranous vocal fold.

DISCUSSION

These data confirm the clinically observed finding that AA produces significant medial displacement of the paralyzed vocal fold. After bilateral AA, there was only 0.8 mm between the vocal process markers, indicating nearly complete closure of the interarytenoid gap.

However, the results do not support the notion that AA results in significant lengthening of the paralyzed vocal fold. Two explanations are possible for the apparent discrepancy between the clinical and experimental findings.

First, measurements made in cadaver preparations must be applied to living human subjects with caution. For example, most patients with clinical laryngeal paralysis do not have complete denervation of the larynx; hence, there may be forces exerted by residual or reinnervated muscle fibers.⁹ The extent of the original injury varies, and reinnervation is a random process; therefore, the effects of these forces would not be expected to result in consistent trends. A more relevant concern is a concept that is generally accepted, but not well documented or specified: that actions of extrinsic laryngeal muscles can have a significant influence on the glottic opening.¹⁰ Thus, AA in vivo and AA ex vivo may result in different spatial changes.

Another possibility is that vocal fold paralysis and AA result in vocal fold rotation out of the visual plane, so that the projection of the vocal fold on the endoscopic image changes. Assessment of clinical images demonstrates that the visual plane of endoscopically acquired images rarely corresponds to the true plane of the glottis. Videoendoscopy generally views the larynx from a relatively posterior aspect in order to expose the anterior commissure. This view displays the anterior tracheal wall, rather than the tracheal lumen or posterior wall. As a consequence, the posterior glottis is closer than the anterior commissure to the eye or camera, and the vocal fold is foreshortened in this view. Caudal motion of the vocal process moves the posterior end of the membranous vocal fold toward the plane of the anterior commissure, reversing some of the visual foreshortening so that the image appears longer. The magnitude of apparent lengthening would depend not only on the distance of inferior displacement, but also on the angle formed by the visual and actual planes in the starting position. For example, if the angle of the visual plane with the vocal fold is 60° and the vocal fold length is 12 mm, then the projection would be 6 mm. A 3-mm drop of the vocal process would increase the length of the projection to about 9.7 mm — a greater than 50% increase. On the other hand, if the initial angle of the vocal fold with the visual plane is only 30°, then the projection of a 12-mm fold would be about 10 mm, and a 3-mm drop would increase the projection only to about 11.5 mm — a length increase of only 15%.

The present data are consistent with previous observations that the vocal process elevates with abduction and moves caudally with adduction.^{6,11,12} Differences between the vertical levels of the paralyzed vocal folds are felt to be responsible for the severity of vocal symptoms in some patients with a vocal fold paralyzed in a relatively lateral position. However, in these cases the paralyzed vocal fold is reported to be

lower than the normal fold.¹³ Blitzer et al¹⁴ noted that in patients with poorer voice quality, the arytenoid cartilage is tipped anteriorly and the vocal fold is at a slightly different level than the normal one. Anterior "sagging" of the arytenoid would result in inferior displacement of the vocal process, and consequently, a vocal fold lower than the normal one. Such distortion could be attributed to the loss of posterior support from the posterior cricoarytenoid muscle. The posterior cricoarytenoid muscle has been shown to be tonically active and to contract during phonation, and therefore is likely to provide posterior traction on the arytenoid in the normal, living subject.¹⁵ Thus, the "cadaveric" position of the vocal fold would be outside the normal physiologic trajectory of motion. This is not impossible, as the cricoarytenoid joint is multi-axial.¹¹

The issue of the vertical level of the paralyzed vocal fold is not trivial. If the paralyzed vocal fold indeed lies below its normal mate, as reported in some patients, then AA as performed in this experiment could increase that difference. Any improvement gained by medialization would be offset to some degree by exacerbation of the level difference. On the other hand, if the vocal process is above the glottal plane, as in the cadaver, then the inferior displacement achieved by AA would be beneficial.

Vocal fold position in patients with paralysis is not necessarily the same as that in the cadaver, and in fact, there is probably significant variation in position within patients. In the living patient, the larynx is subject to a number of forces due to the action of extrinsic muscles, contralateral intrinsic muscles, and the bilaterally innervated interarytenoid muscle and traction by the trachea and pharynx. In addition, the "paralyzed" muscles usually have various degrees of persisting or regenerated innervation.

A second implication of the variable forces at work in the larynx is that the mechanical effects of the AA in excised cadaver larynges may not reflect the actual results in patients. Moreover, the precise placement of sutures in clinical surgery is variable.

The results of these cadaver studies provide information that will be useful in the design of future clinical studies. To determine the appropriateness of the displacement achieved by AA, the real issue to be determined is the spatial relationship of the normal and paralyzed vocal processes during phonation in living subjects. This information would define the ideal configurational change for surgical rehabilitation. In vivo studies of vocal fold level in patients with laryngeal paralysis, before and after surgery, are necessary to clarify these issues.

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ENDOSCOPIC MANAGEMENT OF A COMBINED LARYNGOCELE

BRIAN J. SZWARC, MD

HASKINS K. KASHIMA, MD

BALTIMORE, MARYLAND

A 70-year-old woman presented with hoarseness, foul sputum, and a soft neck mass. Clinical and radiographic examination disclosed findings consistent with a combined internal and external laryngopyocele. Antibiotics, throat irrigations, and warm packs applied to the neck resulted in full resolution of the neck mass and subtotal regression of the supraglottic swelling. Endoscopic vestibulectomy was performed with a carbon dioxide laser; neither residual neck mass nor a tract leading to the extralaryngeal neck swelling was detected. The immediate and long-term clinical course has been uneventful. This report is the first description of the definitive endoscopic management of a combined laryngocele. The pathogenesis of the laryngocele and the rationale and technique for endoscopic management are discussed.

KEY WORDS — endoscopy, laryngocele, laser, vestibulectomy.

INTRODUCTION

Laryngoceles are symptomatic, air-filled dilations of the saccule, a blind sac extending cephalad within the false vocal fold. A standard classification differentiates laryngoceles into internal, external, and combined. Laryngocele incidence is reported to be 1 per 2.5 million population per year without a left or right preponderance.¹ Presentation occurs most commonly in white men in the fifth or sixth decade of life. Acquired laryngoceles may represent slow enlargement of the saccule. Patients with occupations or habits involving prolonged periods of increased pressure within the laryngeal lumen, such as drill sergeants, wind instrument players, and frequent shouters, may be predisposed.² Congenital laryngoceles occur rarely and may cause airway compromise in newborns.

Anatomy and Definitions. The laryngeal ventricle, *ventriculus laryngis* (Morgagnii), is a fusiform fossa bounded inferiorly by the true vocal fold and superiorly by the vestibular (or false) vocal fold. The saccule develops within the false vocal fold. The anterior roof of the ventricle leads to a cecal pouch lined by ciliated mucous membrane, called the saccule (*appendix ventriculi laryngis*; *sacculus laryngis*). The lining mucous membrane has 60 to 70 mucous glands functioning to lubricate the surface of the vocal folds. A saccular cyst is a mucus-filled dilatation distinguishable from the saccule in that it is isolated from the larynx and does not contain air.² Laryngoceles are air-containing dilations of the saccule that communicate with the laryngeal lumen and may become temporarily distended with mucus. The internal laryngocele remains confined within the

false (vestibular) vocal fold. An external laryngocele is an extension of an internal laryngocele, with which it retains continuity via the thyrohyoid membrane at the site of the superior laryngeal neurovasculature axis. Coincidence of internal and external laryngoceles is termed a combined laryngocele (Fig 1²).

Pathogenesis. Saccular disorders form an interrelated spectrum described by Holinger et al² and DeSanto³ in their respective comprehensive publications. Saccular variables include size, presence or absence of communication with the laryngeal lumen, and infection.³ The presence or absence of symptoms distinguishes a laryngocele from a large saccule; symptoms are lacking in the normal saccule. Secondary infection of a laryngocele produces a laryngopyocele (Fig 2³).

Recognition and Detection. The clinical symptoms of internal and external laryngoceles are dis-

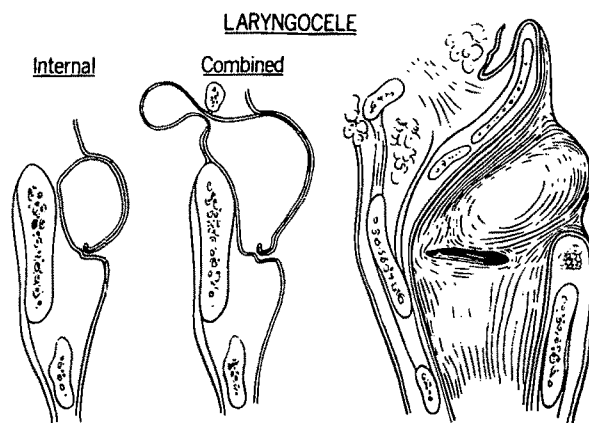


Fig 1. Combined and internal laryngoceles. (Reprinted by permission.²)

From the Department of Otolaryngology-Head and Neck Surgery, Johns Hopkins Hospital, Baltimore, Maryland.

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CORRESPONDENCE — Haskins K. Kashima, MD, Johns Hopkins Hospital, Dept of Otolaryngology-Head and Neck Surgery, JHOC 6163, 660 N Wolfe St, Baltimore, MD 21287.

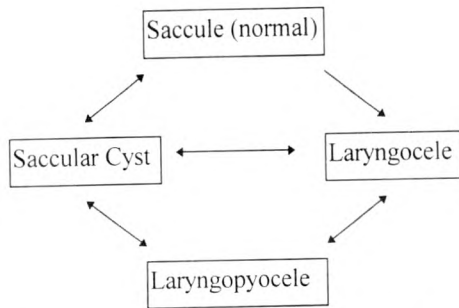


Fig 2. Interrelations among saccular disorders. (Modified from DeSanto.³)

tinct. Internal laryngoceles may interfere with phonation or the airway and most commonly present with hoarseness, stridor, foreign body sensation, sore throat, snoring, or cough. They can be visualized within the larynx as cystic swellings of the vestibular fold. External laryngoceles may present as a neck mass with or without laryngeal symptoms. The lesion overlies or is near the thyrohyoid membrane and is lateral to the thyroid cartilage, usually anterior to the sternocleidomastoid muscle.³ The infected laryngocele may present as a neck abscess. A laryngocele is suspected from clinical findings, confirmed by computed tomography (CT) and magnetic resonance imaging (MRI) examinations, and verified at laryngoscopy.

CASE REPORT

Right neck swelling accompanied by progressive hoarseness of several months' duration was first

noted in a 70-year-old white woman following a motor vehicle accident. Examination disclosed an asymptomatic right neck swelling, noninflamed enlargement of the ipsilateral vestibular fold, bilateral vocal polyps, and normal vocal fold motion. The MRI showed a lobulated 3×3×2-cm low-signal right neck mass. An internal laryngeal component was continuous through the thyrohyoid membrane, with the external component located deep to the strap muscles and platysma (Fig 3).

Intensive treatment with intravenous antibiotics, warm throat irrigations, and the cessation of the patient's 2-pack-per-day smoking habit produced resolution of symptoms over a several-week period. Follow-up office examination and repeated MRI revealed complete resolution of the external neck mass. The internal laryngocele persisted as swelling within the vestibular fold, causing a thickened aryepiglottic fold and expansion of the lateral pharyngoepiglottic fold. Vocal fold motion was intact, with dampening of the mucosal wave presumably due to a mass effect of the cyst.

Eight weeks after the initial presentation, a suspension laryngoscopy and internal laryngocele excision by carbon dioxide (CO₂) laser vestibulectomy was performed. A 3-cm smooth, cystic swelling and an expanded right supraglottic compartment were found. There was no palpable thickening or mass at the right thyrohyoid membrane, the site of the original neck presentation. The laryngocele contained a



Fig 3. Magnetic resonance imaging scan of bilobed laryngocele showing internal and external components (right) and isthmus (left).

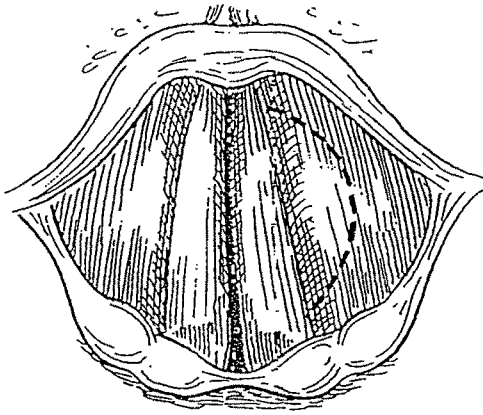


Fig 4. Vestibulectomy incision. (Reprinted by permission.⁴)

white, creamy fluid; culture showed no growth. No isthmus was identified leading to the external laryngocele, and palpation of the neck did not express any abnormal secretions into the excision site. The patient did well and was discharged on postoperative day 1. The patient has been in follow-up for 5 years without evidence of recurrence, documented by clinical and MRI examinations. The external portion of the laryngocele has resolved completely. Her voice is reportedly better than it has been in years. Pathology described a benign cyst lined by pseudostratified ciliated epithelium.

Laryngocele Treatment Options. Treatment of laryngoceles is surgical and varies with the size and type of lesion. The approach may be endolaryngeal or external through the neck. The external approach is generally accepted as the treatment of choice for large internal laryngoceles, combined laryngoceles, and external laryngoceles. The superior margin of the thyroid cartilage is usually excised to expose and access the paraglottic space.

Endoscopic excision by CO₂ laser vestibulectomy avoids potential damage to the superior laryngeal neurovasculature and has less operative morbidity than the external approach. Small internal laryngoceles are amenable to this type of treatment. Endoscopic operation in larger laryngoceles may achieve subtotal excision (marsupialization), leaving a portion of the cyst in the lateral ventricle. Aspiration has been suggested as a minimally invasive procedure; however, recurrence is common, with multiple operations required at times.

Vestibulectomy. Vestibulectomy, excision of the vestibular fold, has been used in the diagnostic and definitive management of early glottic carcinomas. Vestibulectomy completely excises the internal laryngocele and provides excellent intraoperative and postoperative exposure. Vestibulectomy is performed after intubation with a laser endotracheal tube, and

the laryngoscope is positioned for optimum exposure of the vestibule ipsilateral to the laryngocele. Moistened cottonoid pledgets are placed in the ventricle to protect the upper surface of the vocal fold from laser impact. The vestibular fold is retracted medially to facilitate access to the intended incision line. Vestibulectomy begins at the vestibuloepiglottic sulcus, superior and lateral to the anterior commissure, and ends at the posterior limits of the laryngeal ventricle (Fig 4⁴). Bleeding from the branches of the superior laryngeal vessels, when encountered, is controlled by tamponade or application of a defocused CO₂ laser. The excised specimen is oriented so that histopathologic examination of the laryngocele, and particularly the neck, can be performed to ascertain that an occult tumor is not overlooked.

Postoperative care consists of antibiotics and, optionally, a short course of steroids. Patient discharge normally occurs on the morning following surgery.⁵ Voice, airway, and swallow are preserved without residual defects. Postoperative visualization of the lateral ventricle is excellent.

DISCUSSION

The laryngocele has traditionally been managed through an external approach. This may be a more invasive and complex procedure than is necessary for many laryngoceles. Work has been done to address laryngoceles endoscopically with success and a lower morbidity.⁶ We propose a more anatomically complete endoscopic procedure, vestibulectomy, which removes the saccule and its commensurate potential space.

Laryngocele management objectives are fourfold. The first objective is to determine the extent (internal or external) and nature (laryngocele or laryngopyocele) of the lesion. Clinical laryngoscopy and CT or MRI examination are necessary to make these precise determinations.^{7,8} The second objective is to choose the method of treatment; ideally, the epithelium-lined lesion should be excised. The goal is to remove or drain the lesion so as to prohibit cyst recurrence or infection. Traditionally, the internal laryngocele has been addressed endoscopically, and the combined laryngocele, by lateral laryngotomy. In the case described, the laryngocele's internal component was excised and the external component drained, both endoscopically. The third objective is to verify the laryngocele histologically, which is important because of the well-supported association of laryngoceles and carcinoma. The neck of the laryngocele must be examined to ascertain that no neoplastic process is present. The fourth treatment objective is to maximally preserve the voice, airway,

and swallowing, as well as to reduce morbidity.

The minimally invasive endoscopic operation, vestibulectomy, achieved these objectives. Vestibulectomy for management of the internal laryngocele is based on sound principles and experience with vestibulectomy in varied clinical settings.⁵ It offers definite advantages over both endoscopic unroofing and the traditional external approach. Internal laryngocele recurrence is eliminated with vestibulectomy. Vestibulectomy reduces operative time, avoids superior laryngeal nerve and vascular damage, and speeds recovery with less morbidity. Patients must be selected with care: not all laryngoceles, especially external, can be expected to respond to this treatment modality. The present case extends the use of this procedure to curing a combined laryngocele. The external portion was not explored or excised. It was effectively drained into the laryngeal lumen by the vestibulectomy after vigorous medical management with antibiotics, warm compresses, and cough suppression. Operative morbidity was minimal and full

recovery was swift, with no recurrence for over 5 years as of the time of this report.

CONCLUSION

Laryngoceles are uncommon entities that often present as masses within the larynx and/or neck. This case report has outlined a minimally invasive endoscopic procedure, the vestibulectomy, as a treatment option for a combined laryngocele. The intent was to describe its rationale, technique, and outcome. In principle, a bicompartimental abscess can be managed by excision of the internal component with endolaryngeal incision and drainage of the external component. This conceptually has the risk of imperfect management of the external portion. However, in nonacute patients, vestibulectomy has minimal risk and may avoid external exploration altogether. Vestibulectomy can be the definitive and preferred method of cure, provided that the operation is performed after elimination of residual or potential infection and resolution of the external component.

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RECONSTRUCTIVE MICROSURGERY IN HEAD AND NECK CANCER

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EPIGLOTTIC POSITION AFTER CRICOTHYROIDOTOMY: A COMPARISON WITH TRACHEOTOMY

JESSICA W. LIM, MD

PATRICIA KERMAN LERNER, MA

STEPHEN G. ROTHSTEIN, MD

NEW YORK, NEW YORK

Dysphagia is a known problem in patients with tracheotomy, but its association with cricothyroidotomy is not well studied. The purpose of this study was to evaluate dysphagia in patients with cricothyroidotomy and to determine if there is a reliable indicator of swallowing dysfunction in these patients. A review of charts for patients with modified barium swallow studies conducted at the New York University Medical Center Swallowing Disorders Center yielded three groups of patients: patients with cricothyroidotomy, patients with tracheotomy, and normal patients. There were 8 patients in each group. In all patients in the cricothyroidotomy group, there was a greater impairment of epiglottic displacement, laryngeal elevation, and upper esophageal opening than in the tracheotomy group. This problem with epiglottic displacement produced susceptibility to laryngeal penetration and, in turn, increased the risk of aspiration in those patients with cricothyroidotomy. After cricothyroidotomy tube removal, a return to normal epiglottic movement was observed within 2 months. One mechanism of swallowing dysfunction is impaired posterior displacement of the epiglottis over the glottic aperture. This impaired epiglottic motion appears to be related to restricted laryngeal elevation secondary to tethering of the larynx anteriorly at the site of the cricothyroidotomy. Additionally, we noted a decrease in the opening of the upper esophageal sphincter.

KEY WORDS — aspiration, cricothyroidotomy, dysphagia, epiglottis, swallowing, tracheotomy, upper esophageal sphincter.

INTRODUCTION

The presence of a tracheotomy may produce a deleterious effect on swallowing.¹ In a review by Stauffer et al,² 38% of patients with tracheotomy complained of dysphagia. This effect is probably related to failure of elevation and anterior rotation of the larynx, a decrease in epiglottic closure over the laryngeal vestibule, and a decrease in cricopharyngeal opening.³

The effect of cricothyroidotomy on swallowing has not been well studied. Previous reviews of complications associated with cricothyroidotomy have focused on subglottic stenosis and voice changes.⁴⁻⁸ That dysphagia is rarely mentioned may be due to the type of reviews conducted rather than its true infrequency. We undertook this study to gain insight into the effect of cricothyroidotomy on swallowing and to determine if there are specific related findings on modified barium swallow study, also known as the cine-esophagram.

METHODS

The medical records of all patients seen at the New York University Medical Center Swallowing Disorders Center from 1992 to 1995 were reviewed. Patients selected for inclusion in this study had undergone tracheotomy (8 patients) or cricothyroidotomy

(8 patients) and were referred to the Center for dysphagia. These patients underwent their procedures for airway management and pulmonary toilet. Patients with vocal cord impairment or other neurologic problems were excluded. A control group of 8 patients with complaints of dysphagia who subsequently had normal findings on modified barium swallow study was chosen. This group was matched for age and concurrent medical problems (ie, coronary artery disease) with the other two groups. For the three groups, ages ranged from 60 to 81 years. There were 12 men and 12 women.

The cricothyroidotomies and tracheotomies were performed in the standard fashion. The cricothyroidotomies were performed by cardiovascular surgeons on postoperative cardiac patients. The tracheotomies were performed by the otolaryngology service. Shiley No. 8 plastic high-volume, low-pressure cuffed tubes were used for every patient. All procedures were performed for prolonged ventilator support or pulmonary toilet.

The modified barium swallow studies of the selected patients were reviewed, and the following three parameters were evaluated: 1) epiglottic displacement, 2) laryngeal elevation, and 3) upper esophageal sphincter (UES) opening. Each parameter was graded as normal, partial, or none (see

From the Departments of Otolaryngology (Lim, Rothstein) and Rehabilitation Medicine (Lerner), New York University Medical Center, New York, New York.

Presented at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 6-7, 1996.

CORRESPONDENCE — Stephen G. Rothstein, MD, Dept of Otolaryngology, New York University Medical Center, 530 First Ave, Suite 3C, New York, NY 10016.

MODIFIED BARIUM SWALLOW STUDY

	Cricothyroidotomy Group (No. of Pts)	Tracheotomy Group (No. of Pts)
Epiglottic displacement		
Normal = full movement over laryngeal vestibule	0	7
Partial = incomplete movement over laryngeal vestibule	2	1
None = no movement over laryngeal vestibule	6	0
Laryngeal elevation		
Normal = complete elevation (2 cm) of larynx	0	5
Partial = incomplete elevation (<1.5 cm) of larynx	2	3
None = no elevation of larynx	6	0
Upper esophageal sphincter (UES) opening		
Normal = complete opening of UES	1	8
Partial = incomplete opening of UES	0	0
None = no opening of UES	7	0

Table).

The modified barium swallow study was performed in the standard fashion, as described by Logemann,⁹ on a lateral fluoroscopic view. The studies were performed with the Shiley tracheotomy tubes in place and with the cuffs deflated. Follow-up ranged from 1 to 24 months.

RESULTS

Patients in the normal group had normal parameters of swallowing. The tracheotomy and cricothyroidotomy groups were compared to the normal group, as well as to each other. The results of the modified barium swallow studies of the cricothyroidotomy and tracheotomy patients are summarized in the Table.

Follow-up was available for all patients. Of the 8 patients with cricothyroidotomy, 5 were decannulated, 1 was converted to a tracheotomy, and 2 died with their tubes in place. Decannulation times ranged from 3 to 6 weeks after the procedure. Of the 5 who were decannulated, all had resumption of normal swallow activity on modified barium swallow study after stoma closure. (Previous studies showed no epiglottic displacement, no laryngeal elevation, and a closed UES.) The patient who had been converted from cricothyroidotomy to tracheotomy died with the tracheotomy tube.

Of the 8 patients with tracheotomy, all had been successfully decannulated by 12 weeks after the procedure. After decannulation, the 8 patients had documented normal swallows. At the time of swallow studies done with tracheotomy, 3 of the 8 patients demonstrated partial laryngeal elevation, normal epiglottic displacement, and normal UES opening.

DISCUSSION

The basic components of the pharyngeal swallow

include velopharyngeal closure, pharyngeal peristalsis, laryngeal closure, anterior movement and elevation of the larynx, and opening of the UES.¹⁰ Anterior and superior movement of the larynx has been found to be the major component of the pharyngeal phase of swallowing. A reduction in elevation prevents base of tongue movement, considered the primary generating force propelling the bolus through the pharynx, and displacement of the epiglottis. Epiglottic movement is further governed by bolus pressure and downward muscular pull. Laryngeal elevation pulls the cricoid cartilage away from its position against the posterior pharyngeal wall and thus accounts for a large proportion of the cross-sectional opening area of the UES.

Nash¹¹ has divided the impact of tracheotomy on swallowing into mechanical and physiological elements. Mechanical elements include reduced laryngeal elevation, the incision, the weight of the tube, and cuff inflation. Disruption of airway pressure and redirection of glottic airflow, as well as blunting of reflexive cough and the glottic closure reflex, are physiological changes produced by the presence of a tracheotomy. In addition, the tracheotomy tube cuff may cause esophageal obstruction, inducing overflow penetration and aspiration. The chronic upper airway bypass caused by tracheotomy may produce dyscoordinated laryngeal closure.

Although both tracheotomy and cricothyroidotomy had effects on all three parameters of swallowing evaluated in this study, the cricothyroidotomy appears to have produced a more significant change.

The higher position of the cricothyroidotomy in the neck further prevents base of tongue movement and laryngeal elevation. Displacement of the epiglottis was significantly impaired, causing the epiglottis to assume a position intermediate in closure or preventing any movement at all, leaving the airway

unprotected. This finding was consistent in all patients in this group.

The cricothyroidotomy tube, in its position between the thyroid cartilage and the cricoid cartilage, widens the angle between the two cartilages. This prevents anterior movement of the cricoid and displaces it inferiorly. The posterior edges of these cartilages would be driven together, further decreasing UES opening.

The UES opening would also be impaired by the cricothyroidotomy's maintaining the cricoid cartilage posteriorly, both by the curve of the tube and by the ties around the neck in that area. This restriction prevents and delays UES opening, creating pyriform residuals and possible overflow into the laryngeal vestibule.

Modified barium swallow study has emerged as a

reliable and informative method for evaluation of swallowing. The fluoroscopic view may be limited by patient anatomy, such as the shoulders' compromising the view of the UES and glottic larynx. Epiglottic movement is an easily identifiable event that may serve as an indicator of swallowing function.

This study is small in terms of numbers of patients, but several trends were evident. Cricothyroidotomy appears to cause consistent problems with epiglottic displacement over the laryngeal vestibule, laryngeal elevation, and UES opening. These changes, particularly the epiglottic position during swallowing, were more marked in the cricothyroidotomy group than the tracheotomy group. Although the patients studied may represent a skewed group, since all were referred with dysphagia, the impairment of the pharyngeal phase of swallowing does not appear to be attributable to causes other than the cricothyroidotomy or tracheotomy.

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INTERAMERICAN ASSOCIATION OF PEDIATRIC OTORHINOLARYNGOLOGY

The biannual meeting of the Interamerican Association of Pediatric Otorhinolaryngology will be held in Sao Paulo, Brazil, September 21-24, 1997. For information, contact Office of the Secretary, Tania Sih, MD, PhD, Rua Itapeva, 366, Suite 102, 01332-000, Sao Paulo SP, Brazil; telephone 55 11 283-4645 or 283-3396; fax 55 11 826-9652 or 542-6037.

KAPOSI'S SARCOMA OF THE LARYNX

NEIL F. SCHIFF, MD

WATERBURY, CONNECTICUT

PEAK WOO, MD

NEW YORK, NEW YORK

DONALD J. ANNINO, MD, DMD

BOSTON, MASSACHUSETTS

STANLEY M. SHAPSHAY, MD

BOSTON, MASSACHUSETTS

Kaposi's sarcoma (KS) is a neoplastic vascular disorder, classically arising in the skin of the lower extremities. As a consequence of the acquired immunodeficiency syndrome (AIDS) epidemic, an increasing number of patients have been found to have KS. In AIDS patients, KS appears to exhibit a more diffuse nature and frequently affects the head and neck. Mucosal lesions are most often seen, commonly involving the oral cavity. Only rare cases of laryngeal involvement have been recorded in the literature. We report 2 cases of KS of the supraglottic larynx. Our first patient, an elderly man of Mediterranean descent, complained of voice change and throat discomfort. Endoscopy with biopsy for diagnosis allowed conservative treatment with chemotherapy. Our second patient was a younger man with AIDS who presented with symptoms of airway obstruction. Management with carbon dioxide laser epiglottectomy was successful in relieving that patient's symptoms. Although rare, KS may present in both healthy and immunocompromised patients, and must be considered in the differential diagnosis of all violaceous lesions of the larynx.

KEY WORDS — acquired immunodeficiency syndrome, Kaposi's sarcoma, larynx.

INTRODUCTION

In 1872, Moricz Kaposi, a Hungarian dermatologist, first described the disease that now bears his name and consists of multiple idiopathic sarcomas of the skin.¹ He characterized the tumors as violaceous, macular cutaneous lesions with irregular borders, classically affecting the lower extremities of elderly Mediterranean and Jewish men. More recently, however, there has been a dramatic rise in the incidence of Kaposi's sarcoma (KS) associated with the acquired immunodeficiency syndrome (AIDS) epidemic. In 1981, KS was incorporated into the Centers for Disease Control diagnostic criteria for AIDS.² Nearly 40% of patients with AIDS will develop KS at some stage in the illness.³

Kaposi's sarcoma of the head and neck has been well described in the literature. Abemayor and Calcaterra,⁴ in 1983, reported 45 cases of KS in AIDS patients, 40% of whom had head and neck manifestations of the disease. The most common sites noted were dermal (44%), oropharyngeal (39%), and cervical lymph node (33%). Oral lesions, described by Goldberg² in 1993, were seen in 71% of 134 patients; the most common sites were the palate (53%), oropharynx (13%), and gingiva (11%).

However, KS rarely involves the larynx, with a review of the literature yielding fewer than 30 cases. The epiglottis appears to be the most common laryn-

geal site, and KS here is nearly always associated with classic skin lesions.⁵ We report 2 more cases of KS involving the larynx, in addition to summarizing the pertinent literature.

CASE REPORTS

Case 1. A 71-year-old man of Italian descent presented complaining of dysphagia and a globus sensation of 3 months' duration. He denied respiratory difficulty, hemoptysis, or weight loss, and had no significant history of alcohol or tobacco use. Indirect laryngoscopy revealed a thickened, dark, purple-blue submucosal lesion involving the free edge of the epiglottis. There was no ulceration. The airway was patent, without evidence of obstruction. The remainder of the findings on head and neck examination were normal.

Suspension microlaryngoscopy was performed, and a biopsy specimen was taken with a carbon dioxide laser. Pathology confirmed KS. The patient was referred to the oncology department, and a single skin lesion on the left hand was noted. Systemic chemotherapy was initiated, which resulted in complete resolution of the lesions as well as the patient's symptoms. The patient remained free of disease until he was lost to follow-up 6 months postoperatively.

Case 2. A 44-year-old man with a 2-month history of mild odynophagia and a muffled voice presented

From the Department of Otolaryngology-Head and Neck Surgery, New England Medical Center, Tufts University School of Medicine, Boston, Massachusetts.

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CORRESPONDENCE — Neil F. Schiff, MD, 171 Grandview Ave, Suite 204, Waterbury, CT 06708.

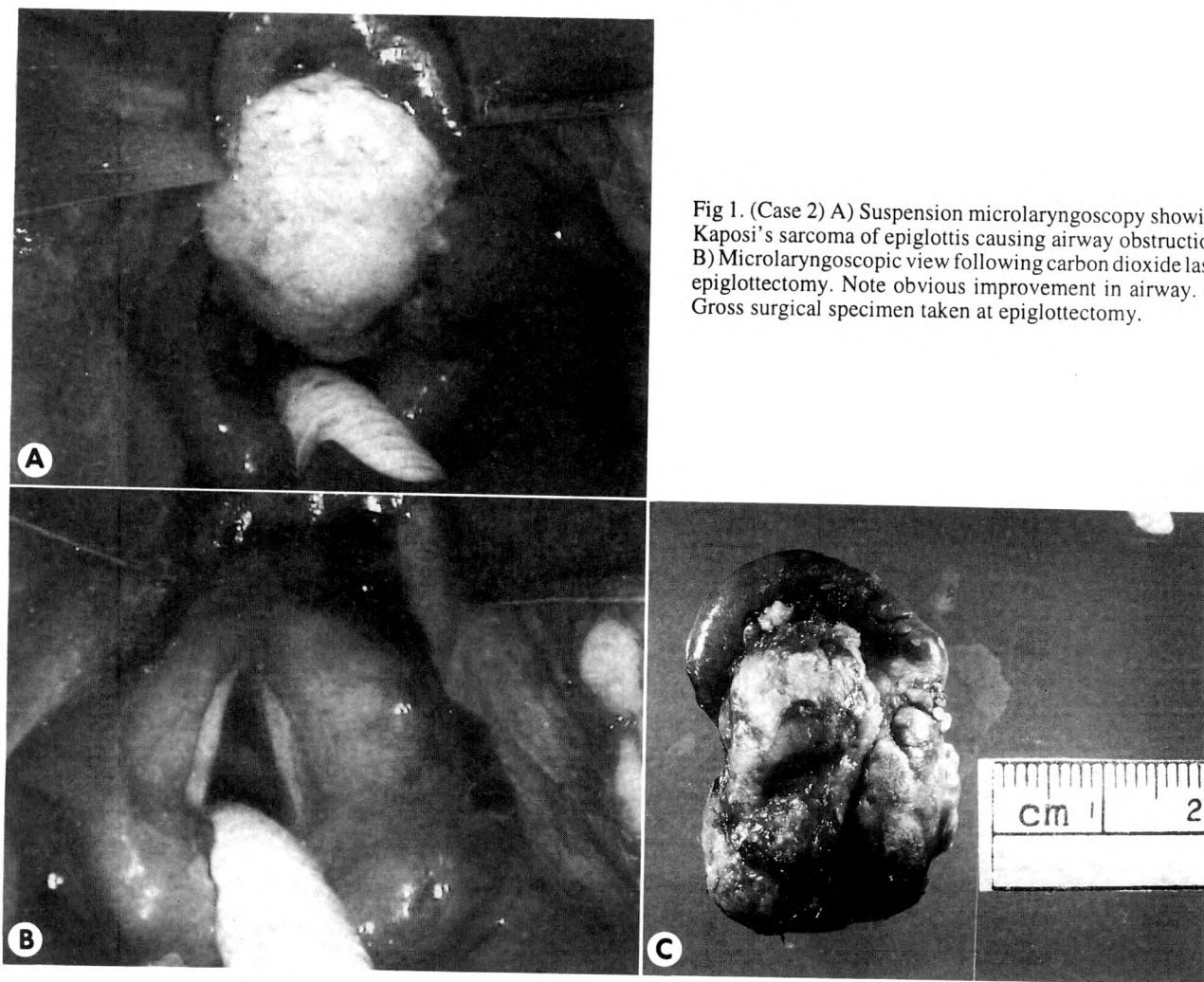


Fig 1. (Case 2) A) Suspension microlaryngoscopy showing Kaposi's sarcoma of epiglottis causing airway obstruction. B) Microlaryngoscopic view following carbon dioxide laser epiglottectomy. Note obvious improvement in airway. C) Gross surgical specimen taken at epiglottectomy.

with worsening symptoms of dyspnea on exertion and dysphagia to both solids and liquids. His past medical history was remarkable for a diagnosis of AIDS, which was made 4 months prior to admission on the basis of the development of *Pneumocystis carinii* pneumonia and a positive human immunodeficiency virus titer.

On physical examination, the patient had inspiratory stridor without acute respiratory distress. A violaceous, nonblanching external nasal lesion, as well as similar multiple upper and lower extremity lesions, was noted. There was bilateral cervical lymphadenopathy. Fiber-optic nasopharyngolaryngoscopy revealed a large, irregular purple lesion involving the epiglottis and obstructing a direct view of the glottis. There was a central area of yellow necrotic debris. The patient was admitted to the hospital for airway management with intravenous hydration, steroids, and antibiotics, as well as cardiopulmonary monitoring. Suspension microlaryngoscopy was performed with a bivalved supraglottic laryngoscope (Fig 1A). An epiglottectomy by means of a carbon dioxide laser was completed without difficulty (Fig 1B,C).

Postoperatively, the patient was much improved, with complete resolution of his stridor and shortness of breath. His voice and swallowing were normal. At 9 months' follow-up, the patient had remained free of symptoms of upper airway obstruction and voice change. Repeat fiber-optic examination revealed a well-healed mucosal surface and a widely patent upper airway. He still, however, suffered from diffuse skin KS, despite repeated cycles of systemic chemotherapy as well as local radiotherapy.

DISCUSSION

Kaposi's sarcoma is considered by most to be a vascular endothelial cell neoplasm of uncertain origin.^{4,6,7} Some, however, suggest that the disease is not a true neoplasm. Rather, it has been postulated to be a reactive condition with multifocal vascular proliferation stimulated by angiogenic factors released by CD4 lymphocytes or viral triggers.^{2,3} Kaposi's sarcoma is currently classified into three separate subtypes with very different clinical behaviors. These are described as the classic, endemic, and epidemic forms² (see Table).

CLASSIFICATION OF KAPOSI'S SARCOMA

	<i>Classic</i>	<i>Endemic Cutaneous</i>	<i>Endemic Lymphadenopathic</i>	<i>Epidemic</i>
Population	White male, Mediterranean or Jewish	Black male, equatorial Africa	Black male, equatorial Africa	Immunocompromised
Age (y)	50-70	25-40	2-13	As per primary diagnosis
Male-female ratio	10-15:1	15:1	3:1	As per primary diagnosis
Clinical course	Indolent	Sometimes aggressive	Aggressive	Aggressive
Prognosis	Good	Good	Poor	Poor

Adapted from Goldberg.²

Classic KS occurs in the older Mediterranean and Jewish populations, with men affected 10 to 15 times more often than women.^{2,8} The lesions are mostly localized, blue-purple nodules in the lower extremities. They are indolent and rarely cause morbidity. Survival ranges from 8 to 13 years. Mortality in these patients is secondary to other disorders. Tumors are often radiosensitive and responsive to single-agent chemotherapy.

Endemic KS, common to the African equatorial region, is characterized by a more disseminated and aggressive disease process. Often involving the lymphatic system, spleen, and gastrointestinal tract, the disease can be rapidly fatal, especially in children and young adults.⁴ Treatment is usually with combination chemotherapy.

Epidemic KS occurs in immunocompromised patients, such as those with organ transplants, taking immunosuppressive medications, or with AIDS. The lesions are aggressive and multicentric. Kaposi's sarcoma is the most common malignant tumor in AIDS.² For unknown reasons, KS in AIDS patients is

seen almost exclusively (95% of cases) in the homosexual and bisexual populations.² Viral cofactors, such as Epstein-Barr virus and cytomegalovirus, have been proposed as etiologic agents in cell transformation and proliferation.⁴

Grossly, the lesions are red-purple, multicentric macules or nodules. Color variation, including the colors blue, black, tan, and brown, can occur.^{6,7} The lesions are nonblanching and typically are nontender unless secondarily infected or traumatized.²

Histologically, the distinctive features of KS are spindle-shaped cells with numerous vascular channels. Extravasated erythrocytes, macrophages, and hemosiderin deposits are scattered in a collagen stroma² (Fig 2). A prominent inflammatory infiltrate can be seen. Immunoperoxidase staining is positive for factor VIII antigen.

Historically, KS has only rarely involved the head and neck, and has usually been seen in conjunction with widespread generalized disease.⁹ Likewise, prior to the emergence of AIDS, classic KS of the larynx was an extremely rare entity, with only 14 cases

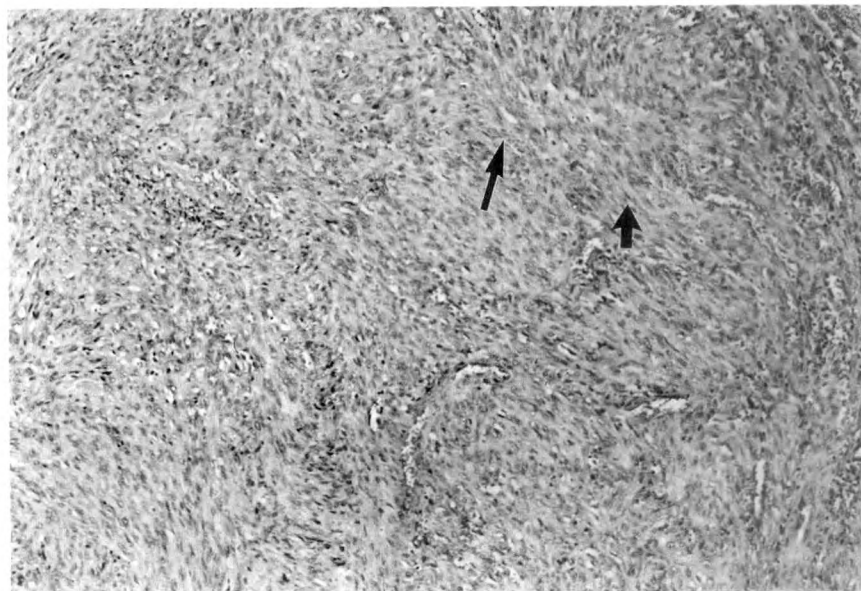


Fig 2. (Case 2) Histomicrograph of Kaposi's sarcoma. Note spindle cells (short arrow) and extravasated erythrocytes (long arrow).

identified in the literature. Reynolds et al,⁷ in 1965, reported KS with involvement of the arytenoids and epiglottis that required an emergent tracheotomy. Coyas et al¹⁰ likewise reported an isolated case of KS of the larynx in a 72-year-old woman treated with tracheotomy followed by radiotherapy.

The largest review in the literature of classic KS involving the larynx was reported by Abramson and Simons⁶ in 1970. They summarized 13 cases of KS that involved the larynx, including the case reported by Reynolds et al.⁷ Most cases were associated with simultaneous involvement of the oropharynx and hypopharynx, as well as with classic skin lesions.⁶

However, with the emergence of AIDS, the incidence of KS has dramatically increased. Nearly 40% of patients with AIDS will develop KS at some stage in the illness, and two thirds of these cases will involve the head and neck region.³ Likewise, laryngeal involvement by KS has been recently reported in increasing numbers of cases, with 15 cases identified in the literature since 1983.

Abemayor and Calcaterra,⁴ in 1983, performed a retrospective review of 18 patients with KS and AIDS, and reported 1 case of epiglottic involvement. Gnepp et al,⁹ in 1984, reported 9 cases of KS of the head and neck, 1 of which involved the anterior commissure of the larynx. The tumor was raised and sessile, and was treated successfully with simple surgical excision.⁹ A second case of laryngeal involvement included an obstructing lesion of the larynx and hypopharynx, treated with tracheotomy and radiotherapy. Initial regression was followed by recurrence and, ultimately, death secondary to diffuse visceral KS and lymphoma.

Weidauer et al,¹¹ in 1986, described a case of KS of the larynx with symptoms including hoarseness, cough, and expectoration of small tissue fragments. Levy and Tansek¹² reported a case of bilateral KS of the true vocal cords associated with diffuse systemic KS and fulminant AIDS. Friedman et al,¹³ in 1996, reported 24 patients with mucosal KS of the head and neck, including 6 patients with KS of the larynx. Patients were treated with intralesional vinblastine sulfate injections, with a partial to complete response rate in over 75% of cases. One patient presented with stridor and multiple laryngeal lesions and required a tracheotomy.¹³

Sporadic cases of KS of the larynx have also been treated with systemic vincristine sulfate or bleomycin sulfate chemotherapy,^{2,14} intralesional vinblastine,¹⁵ and low-dose interferon alfa-2b.¹⁶ Those cases with airway compromise required tracheotomy.^{15,16}

Local and systemic therapies are available for treatment of KS. Local treatment includes surgical excision, radiotherapy, cryotherapy, laser excision, photodynamic therapy, topical tretinoin gel, and intralesional injection of vinblastine.^{13,15} Systemic treatment typically involves single-agent or combination chemotherapy with vincristine, vinblastine, bleomycin, doxorubicin hydrochloride, or etoposide, as well as immunomodulation with interferon- α .^{13,15,16} The superiority of one therapy over the others has not been proven.

Treatment for KS, therefore, is often multimodal and usually aimed at symptomatic relief. Symptoms commonly seen with laryngeal involvement are pain, bleeding, dysphagia, speech abnormalities, and airway compromise. Early treatment is advisable in cases of KS of the larynx to avoid potential airway obstruction and death. Endoscopic excision with a carbon dioxide laser can be readily accomplished for bleeding or obstructing lesions, especially if single or pedunculated. Open excision or complete resection with total laryngectomy is not warranted, since treatment is considered palliative. Mortality in patients with KS only rarely occurs as a direct result of the tumor. More commonly, whether the classic or epidemic form is involved, death occurs secondary to other disease processes.^{5,17} Patients who present with airway obstruction in addition to diffuse or unresectable lesions should undergo tracheotomy followed by systemic therapy.

CONCLUSION

World Health Organization (WHO) statistics for 1995 show nearly 1.2 million cases of AIDS reported worldwide, with estimated total cases greater than 4.5 million.¹⁸ The WHO projections for the year 2000 predict 30 to 40 million people infected with the human immunodeficiency virus, with 10 million cases of AIDS estimated worldwide.

Otolaryngological manifestations of AIDS, though already commonplace, are expected to dramatically increase over the next decade. As a result, KS, which occurs in approximately 40% of AIDS patients, will likewise increase proportionately. Kaposi's sarcoma, while usually presenting with only minor functional and cosmetic problems, can cause significant morbidity and mortality by means of life-threatening airway obstruction and bleeding secondary to laryngeal involvement. It must therefore be included in the differential diagnosis of all pigmented lesions of the larynx. A high index of suspicion must be maintained while a thorough evaluation and appropriate treatment are instituted.

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SELECTIVE COCHLEAR NEURECTOMY FOR DEBILITATING TINNITUS

JACK J. WAZEN, MD
NEW YORK, NEW YORK

DAVID FOYT, MD
LOS ANGELES, CALIFORNIA

MICHAEL SISTI, MD
NEW YORK, NEW YORK

Eighth nerve sections have been performed to control debilitating tinnitus, with various success rates (45% to 76%). Patients with a unilateral profound sensorineural hearing loss and disabling tinnitus perceived in that ear are candidates for such surgery. The concept of a selective cochlear neurectomy with preservation of the vestibular nerve is introduced with two case presentations. The indications for surgery, surgical technique, and results are described. Advantages of preserving the vestibular nerve fibers include the lack of postoperative vertigo and disequilibrium and thus a shorter length of hospital stay, and the conservation of a symmetric vestibular input, obviating the lengthy compensation process that might otherwise be needed, particularly in the elderly. A selective cochlear neurectomy for the control of debilitating tinnitus has proven to be successful in controlling tinnitus in the two patients presented, with the added advantage of preservation of their vestibular function. Further controlled studies are necessary to confirm the advantages and effectiveness of this technique.

KEY WORDS — cochlear neurectomy, tinnitus.

INTRODUCTION

Debilitating tinnitus continues to be a source of significant frustration and depression. The ability to control tinnitus medically continues to evade us. Medications such as the benzodiazepines and antidepressants have been shown to play a limited role in tinnitus control. Biofeedback therapy, acupuncture, cervical alignment by chiropractors, and temporomandibular joint therapy head the list of "alternative medicine" methods desperate tinnitus sufferers have resorted to for an attempt toward relief.

Surgical procedures for the control of debilitating tinnitus have been performed with various success rates. Dandy¹ reported a 50% improvement in tinnitus following eighth cochlear nerve section for Meniere's disease. Fisch² reported a 75% improvement following a cochleovestibular neurectomy (CVN), as compared to 65% following a selective vestibular neurectomy. Silverstein³ also reported a 75% improvement following a CVN, while House and Brackmann⁴ reported 45%, and Gardner,⁵ 48% improvement. Barrs and Brackmann⁶ reported a 62% improvement after CVN, compared to 49% in selective vestibular neurectomies. In a study comparing the tinnitus response in various inner ear procedures, Silverstein et al⁷ reported an overall improvement in 56% of patients, while 76% improved following CVN. Pulec⁸ reported complete relief of tinnitus in 67% of 151

patients following a CVN.

The literature shows evidence suggesting better tinnitus relief with eighth nerve section than with any other procedure. The CVN procedures are reserved for patients who have lost serviceable hearing.

We propose that in the absence of vestibular symptoms and with preserved vestibular responses, a selective cochlear nerve section would yield results similar to those of a CVN without sacrificing vestibular function.

SURGICAL PROCEDURE

The surgical approaches for a selective cochlear neurectomy are similar to the various approaches for a selective vestibular neurectomy, except that the cochlear division of the eighth nerve is sectioned instead of the vestibular division.

The eighth nerve is visualized in the cerebellopontine angle through either the retrolabyrinthine approach or the retrosigmoid approach as described by Silverstein et al.^{9,10} The cleavage plane between the cochlear and the vestibular divisions is identified under high magnification with the operating microscope. A fine, dark line may be seen through the intact nerve surface, or after scoring the perineurium with a fine Beaver eye blade. A fine vascular line is com-

From the Departments of Otolaryngology-Head and Neck Surgery (Wazen, Foyt) and Neurological Surgery (Wazen, Sisti), Columbia University, College of Physicians and Surgeons, New York, New York.

CORRESPONDENCE — Jack J. Wazen, MD, Dept of Otolaryngology-Head and Neck Surgery, Columbia University, 630 W 168th St, New York, NY 10032.

monly seen coursing along the cleavage plane. Once the cleavage plane is identified, the nerves are bluntly separated with a nerve dissector, and the cochlear division is cut with sharp scissors or a blade. The cochlear division is the inferior one, facing the lower cranial nerves, away from nerve V and the tentorium. The facial nerve lies deep to the eighth nerve through this approach and needs to be carefully identified and monitored throughout the procedure.

CASE REPORTS

Case 1. A 49-year-old man presented with severe, constant, debilitating right ear tinnitus of 2 years' duration that had followed a head injury. He had lost his hearing in his right ear, but denied dizziness, vertigo, or disequilibrium. The tinnitus became so intense that he was unable to sleep or work.

Physical examination revealed a normal head and neck, and the results of otological and neuro-otological evaluation were normal. The audiogram showed a profound sensorineural hearing loss in the right ear with no speech discrimination. The left ear was normal. Electronystagmography (ENG) revealed a 30% reduced vestibular response in the right ear. A computed tomography scan of the temporal bones revealed a high-riding sigmoid sinus, and a low transverse sinus with no evidence of a fracture. Magnetic resonance imaging with contrast was negative.

A right retrosigmoid cochlear neurectomy was performed. The patient reported a significant improvement in his tinnitus postoperatively. Postoperative ENG revealed no change in the caloric responses. A 14-month postoperative evaluation revealed the patient to be stable. On a tinnitus severity scale system from 1 to 5, 1 being barely noticeable and 5 being disabling, the patient's rating dropped from a preoperative score of 5 to an immediate postoperative score of 1, where it remained for 1 month, then stabilized at 2, where it has remained for the last year.

Case 2. A 36-year-old woman suffered a sudden right sensorineural hearing loss accompanied by vertigo, nausea, and vomiting. She subsequently recovered her balance but not her hearing. One year later, she still felt debilitated by intense right ear tinnitus. The audiogram revealed a profound right sensorineural hearing loss with no speech discrimination, and ENG showed a 56% reduced vestibular response in the right ear. Magnetic resonance imaging with contrast was negative.

A right retrolabyrinthine cochlear neurectomy was performed, with significant resolution of the tinnitus. The patient suffered no vestibular symp-

toms postoperatively, and her ENG findings remained unchanged. A follow-up evaluation done 10 months postoperatively revealed her to be satisfied with her outcome. Her tinnitus severity grade dropped from 5 to 3. She reported a significant improvement in her ability to work and cope with her tinnitus, and said she would recommend the procedure to a friend under similar circumstances.

DISCUSSION

Surgery for the control of tinnitus is rarely indicated or performed, in comparison to the millions of tinnitus sufferers. Candidates for such surgery must have 1) lost all serviceable hearing in the affected ear, 2) failed to respond to conservative medical management, and 3) accepted the fact that their tinnitus may persist despite surgery.

Only peripherally generated tinnitus is expected to respond to surgery. The most successful procedures have included an eighth nerve section. However, performing a total eighth nerve section for tinnitus destroys the vestibular function unnecessarily. In the absence of vestibular symptoms, a selective cochlear neurectomy denervates the auditory system while saving vestibular function. The success rates of a cochlear neurectomy are not expected to be better than those of the eighth nerve section. The advantages of a selective cochlear neurectomy are earlier ambulation and a reduced length of stay, due to the lack of vertigo and disequilibrium with the preservation of vestibular function.

The ability to separate the vestibular fibers from the cochlear in the cerebellopontine angle has been well documented. Whether the eighth nerve is approached through a retrolabyrinthine or a retrosigmoid exposure has not made a remarkable difference in the vestibular neurectomy success rates.¹¹ The choice is based on anatomic limitations and the surgeon's preference. The advantages of the retrolabyrinthine approach are the shorter distance to the nerve and a lesser need for cerebellar retraction. The advantage of the retrosigmoid approach is faster and wider exposure with less mastoid drilling.

The middle fossa approach, while quite successful for vestibular neurectomies, is less favored for cochlear nerve sections. Reaching the cochlear nerve inside the internal auditory canal through the middle fossa approach requires retraction of the facial and superior vestibular nerves, which increases the risks for facial nerve palsies and vestibular dysfunctions.

The risks and complications of a selective cochlear neurectomy are similar to those of a vestibular neu-

rectomy, including cerebrospinal fluid leaks, meningitis, and facial palsies.

The purpose of this paper is to propose the concept of a selective cochlear neurectomy in controlling debilitating tinnitus, based on the successful outcome of the two cases presented. Obviously, further controlled studies using complete eighth nerve sections and medical management are necessary. These are in progress, using an extensive tinnitus evaluation questionnaire that includes a severity and frequency match-

ing.

CONCLUSION

A selective cochlear neurectomy for the control of debilitating tinnitus has proved to be as successful as a total eighth nerve section in the two cases herein reported, with the added advantage of preservation of vestibular function. The retrolabyrinthine or retrosigmoid approach provides the safest access to the cochlear nerve and avoids facial or superior vestibular nerve retraction.

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AURICULAR AND MIDDLE EAR MALFORMATIONS, EAR DEFECTS AND THEIR RECONSTRUCTIONS

The Third International Symposium on Auricular and Middle Ear Malformations, Ear Defects and Their Reconstructions will be held in Lubeck, Germany, September 6-9, 1997. For more information, contact Dr H. Weerda or Dr R. Siegert, Department for Otorhinolaryngology and Plastic Head and Neck Surgery, Medizinische Universität zu Lubeck, Ratzeburger Allee 160, D-23538 Lubeck, Germany; telephone (0) 451 500 3189; fax (0) 451 500 4192.

INTERACTION BETWEEN ORAL ALPHA-STREPTOCOCCI AND GROUP A STREPTOCOCCI IN PATIENTS WITH TONSILLITIS

ISAO FUJIMORI, MD
IZURU NOZAWA, MD

KAZUHITO KIKUSHIMA, MD
REI GOTO, MD
YAMANASHI, JAPAN

KEN-ICHI HISAMATSU, MD
YOSHIHIKO MURAKAMI, MD

The incidence of oral α -streptococci with inhibitory activity against group A streptococci, as a defense mechanism against bacterial infection in the oral cavity, was investigated in 141 patients with streptococcal tonsillitis. The study population included both children ($n = 79$) and adults ($n = 62$). Infection by group A streptococci appeared to be more common in children than in adults, as the detection rates of inhibitory α -streptococci in healthy children (29.7%), as well as pediatric patients with tonsillitis (14.9%), were lower than those in adults (63.0%; $p < .01$). It is possible to consider oral α -streptococci with inhibitory activity to be among the indications for tonsillectomy in patients with streptococcal tonsillitis, since the detection rate of inhibitory α -streptococci in surgical cases (10.9%) was significantly lower than that in nonsurgical cases (31.1%; $p < .01$). The high detection rate of these strains during the postoperative state supported the observation that the incidence of group A streptococcal infection was decreased postoperatively. Accordingly, it is useful to investigate bacterial interference between oral α -streptococci and group A streptococci in patients scheduled for tonsillectomy.

KEY WORDS — alpha-streptococci, group A streptococci, tonsillectomy, tonsillitis.

INTRODUCTION

Although group A β -streptococci are the major causative agents of bacterial tonsillitis, concern regarding these pathogens has decreased since the development of antibiotics. However, group A streptococcal infection remains dangerous, since an increase in the incidence of the invasive disease known as "toxic shock syndrome"^{1,2} and a resurgence of rheumatic fever in the United States^{3,4} have been reported recently.

Normal oral flora, in addition to mucosal ciliary movement and secretory IgA, play important roles in the nonspecific host defense mechanism against infections of the upper respiratory-digestive tract. Thompson and Shibuya,⁵ Sanders et al,⁶ Sprunt and Redman,⁷ and Dajani et al⁸ have shown that some oral α -streptococci have inhibitory effects on the growth of pyogenic streptococci such as group A streptococci. Brook and Gober⁹ have shown that the absence of interfering α -streptococci and the presence of β -lactamase-producing bacteria is associated with penicillin failure in the treatment of group A β -hemolytic streptococcal pharyngotonsillitis.

In this paper, we present the results of our investigations of the mode of interaction among oral streptococci isolated from patients with tonsillitis and discuss its clinical significance, particularly concerning the determination of indications for tonsillectomy. The absence of inhibitory α -streptococci in throat

cultures may be another indication for tonsillectomy, or at least, an adjunctive indication.

MATERIAL AND METHODS

Between April 1994 and May 1995, throat swabs were obtained from 141 patients with a history of at least 1 streptococcal throat infection confirmed by a positive rapid test for group A streptococcus (Abbott Testpack plus Strep A, Abbott) and a streptococcal throat culture positive for group A streptococci during the previous 2 months. Moreover, all subjects tested were those from whom we isolated both group A streptococci and α -streptococci. Patients were divided into children ($n = 79$) and adults ($n = 62$). Pediatric subjects ranged in age from 2 to 12 years (mean age 6.3), and adults ranged in age from 20 to 68 years (mean age 36.4). Furthermore, both groups were divided by number of episodes of streptococcal tonsillitis within the previous year as follows: subjects ($n = 88$, children = 47, adults = 41) that had 1 to 3 episodes of streptococcal tonsillitis constituted the nonsurgical group, and those ($n = 53$, children = 32, adults = 21) that had more than 4 episodes constituted the group that was treated surgically.

One hundred thirty-six healthy individuals who had no episodes of streptococcal tonsillitis within the previous year were included as controls and divided into two age groups: 62 healthy schoolchildren (ages 7 to 9 years, mean age 7.8) and 74 healthy adults (ages

From the Department of Otolaryngology, Yamanashi Medical University, Yamanashi, Japan.

CORRESPONDENCE — Isao Fujimori, MD, Dept of Otolaryngology, Yamanashi Medical University, 1110 Shimo-kato Tamaho-cho, Yamanashi, 409-38, Japan.

TABLE 1. COMPARISON OF DETECTION RATE OF α -STREPTOCOCCI WITH INHIBITORY ACTIVITY AGAINST GROUP A STREPTOCOCCI BETWEEN CONTROL SUBJECTS AND PATIENTS WITH TONSILLITIS

	Inhibitory Activity	Children		Adults		Significance*
		No.	%	No.	%	
Tonsillitis	Moderate and/or strong	118/790	14.9	219/620	35.3	$p < .01$
	Strong	15/790	1.9	43/620	6.9	$p < .05$
Control	Moderate and/or strong	184/620	29.7	466/740	63.0	$p < .01$
	Strong	26/620	4.2	141/740	19.1	$p < .01$

Inhibitory rate (%) = $\frac{\text{Number of strains with inhibitory activity}}{\text{Number of strains tested}} \times 100.$

*Between children and adults.

20 to 38 years, mean age 28.3). All individuals tested and their families gave their informed consent before participating in this study.

After the patients gargled with sterile saline solution, throat swabs were obtained with Transwabs ENT (Medical Wire Equipment Co, Ltd, United Kingdom) pressed firmly into the tonsillar crypts in the tested individuals, and pressed firmly onto the fossa tonsillaris. Specimens were obtained more than 2 weeks after any medical or surgical treatment, when it was no longer considered an influence.⁸

All swabs were immediately brought to the microbiology laboratory. Throat swabs were placed on the surface of 5% sheep's blood agar plates (trypticase soy, BBL) and cultured aerobically at 37°C for 24 hours. Further, 10 colonies of oral α -streptococci isolated from the primary blood agar plates were streak-cultured for 24 hours at 37°C.

A clinical isolate of *Streptococcus pyogenes* (6-22, T type 12) as an indicator strain was suspended in heart infusion broth (Difco) at a concentration of 10^7 colony-forming units (CFU) per milliliter. Thereafter, the indicator strain was vertically inoculated straight onto the α -streptococci grown on the blood agar plates. Twenty-four hours after inoculation, the *S pyogenes* growth condition was examined.

An assay was considered to indicate moderate inhibitory activity (+) if interference was apparent as an area on only the overlay surface where the growth of the indicator strain and hemolysis had been inhibited. If interference was detected in an area surrounding the overlay surface, the α -streptococcus strain examined was considered to have strong inhibitory activity (++) against the indicator strain.

α -Streptococci with inhibitory activity were selected at random for identification. These strains were examined biochemically by the API 20 Strep System (Bio Merieux SA, France).

Detection rates were compared by χ^2 test, Wilcoxon's signed rank test, and Mann-Whitney U test. Differences at $p < .05$ were considered to be significant.

RESULTS

We examined the isolated oral α -streptococci with inhibitory activity against *S pyogenes* among the patient and control groups (Table 1). In the patients, the number of inhibitory strains isolated from the children was significantly lower than that from adults ($p < .01$). In the pediatric patients, the detection rate of inhibitory strains was lower than that in healthy children ($p < .05$). In adults, the detection rate of inhibitory strains in patients was lower than that in control subjects ($p < .01$).

Next, we investigated the detection of inhibitory α -streptococci by number of episodes of streptococcal tonsillitis (Table 2). The detection rate of inhibitory strains isolated in children who had more than 4 episodes within the previous year was significantly lower than that in children who had no episodes ($p < .01$). However, the detection rate of inhibitory strains in pediatric controls did not differ from that in subjects who had 1 to 3 episodes within the previous year. In adults, the detection rate of inhibitory strains isolated in subjects who had more than 4 episodes within the previous year was the lowest among all groups examined ($p < .01$). The detection rate of inhibitory strains in adult controls differed from that in subjects who had 1 to 3 episodes within the previous year ($p < .01$).

In the surgical group more than 2 weeks after tonsillectomy, the isolation rates of inhibitory strains were increased markedly, not only in children, but also in adults ($p < .01$), and the incidence was approximately 62%, as shown in Table 3. Postoperatively, these strains 1, 2, and 3 months after surgery were detected at similar rates. Detection rates of α -streptococci with strong inhibitory activity also increased postoperatively. However, numbers of patients in whom we detected *S pyogenes* 2 weeks and 1, 2, and 3 months after surgery were 2, 1, 2, and 1, respectively. In such patients, the detection rate of inhibitory α -streptococci did not increase postoperatively.

During biochemical identification of inhibitory strains, approximately 60% of the tested strains with

TABLE 2. COMPARISON OF DETECTION RATE OF α -STREPTOCOCCI WITH INHIBITORY ACTIVITY AGAINST GROUP A STREPTOCOCCI BY FREQUENCY OF TONSILLITIS

Inhibitory Activity		Children		Adults		Significance*
		No.	%	No.	%	
Control	Moderate and/or strong	184/620	29.7	466/740	63.0	$p < .01$
	Strong	26/620	4.2	141/740	19.1	$p < .01$
1-3 episodes	Moderate and/or strong	96/470	20.4	178/410	43.4	$p < .01$
	Strong	13/470	2.8	37/410	9.0	$p < .05$
More than 4 episodes	Moderate and/or strong	22/320	6.9	41/210	19.5	$p < .01$
	Strong	2/320	0.6	6/210	2.9	NS

*Between children and adults.

moderate inhibitory activity consisted of *Streptococcus sanguis*, and 20% consisted of *Streptococcus mitis*. In contrast, approximately 65% of the tested strains with strong inhibitory activity consisted of *Streptococcus salivarius*.

DISCUSSION

Thompson and Shibuya⁵ first reported that some bacteria produce bacteriocins and release them into the extracellular environment. Numerous studies of α -streptococci with inhibitory activity against group A β -streptococci have since been reported.^{6-8,10,11} Various investigations have suggested that the observed inhibition was related to the production of hydrogen peroxide,⁸ reduction of pH,¹² or depletion of nutrients.¹³ Furthermore, researchers have attempted to purify bacteriocins produced by α -streptococci constituting part of the normal pharyngeal flora such as *S sanguis*,^{14,15} *S mitis*,¹⁵ *S salivarius*,¹³ and *Streptococcus mutans*,¹⁶ and these compounds have been named viridin A, viridin B, enocin, and mutacin, respectively.

Although several methods of investigating bacterial interference have been studied, the method that we devised and described here is simpler than those reported previously. Our method allows not only investigation of the inhibitory activities against sev-

eral indicator strains simultaneously, but also discrimination between direct and expansive inhibitory activity.

In schoolchildren harboring β -streptococci, the isolation rate of α -streptococci with inhibitory activity was markedly lower than that in children in whom β -streptococci were not detected in the throat. These results support the suggestion that the decline of inhibitory α -streptococci facilitates colonization by β -streptococci of the tonsils. Furthermore, in children who underwent tonsillectomy, there was significant recovery of inhibitory strains colonizing the throat after surgery. Although the mechanism by which inhibitory strains colonize the throat after tonsillectomy remains obscure, the paucity of cases harboring β -streptococci after the operation suggests that such inhibitory strains play an important role in the normal defense mechanism after throat surgery.

At present, indications for tonsillectomy are closely related to recurrent infection, persistent detection of group A β -streptococci, and positive results on serologic examination for such antibodies as antistreptolysin O and antideoxyribonuclease II. However, we suggest that the results of bacteriologic examination of the throat flora, ie, absence of inhibitory α -streptococci in throat cultures, may be another indication for tonsillectomy, or at least an adjunctive indication.

TABLE 3. DETECTION RATE OF α -STREPTOCOCCI WITH INHIBITORY ACTIVITY AGAINST GROUP A STREPTOCOCCI IN SURGICAL PATIENTS

Inhibitory Activity		Children		Adults	
		No.	%	No.	%
Preoperative	Moderate and/or strong	22/320	6.9	41/210	19.5
	Strong	2/320	0.6	6/210	2.9
Postoperative (1 mo)	Moderate and/or strong	147/320	45.9	120/210	57.1
	Strong	26/320	8.1	32/210	15.2
Postoperative (2 mo)	Moderate and/or strong	131/320	40.9	131/210	62.4
	Strong	29/320	9.1	36/210	17.1
Postoperative (3 mo)	Moderate and/or strong	141/320	44.1	121/210	57.6
	Strong	31/320	9.7	38/210	18.1
Postoperative (6 mo)	Moderate and/or strong	153/320	47.8	124/210	59.0
	Strong	27/320	8.4	35/210	16.7

Accordingly, an active surgical approach and improvement of the normal oral flora is considered to be desirable for children infected with group A streptococci, such as those with recurrent tonsillitis.

In addition, Roos et al^{17,18} combined the incidence of inhibitory α -streptococci with clinical treatment

and reported that treatment of patients with β -streptococcal tonsillitis and/or pharyngitis with antibiotics followed by recolonization with α -streptococci seemed to hinder further recurrence. Accordingly, it is concluded that we should consider selective treatment, ie, not only surgical intervention, but also microbial therapy, for streptococcal infection.

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HEARING LOSS (IN NONOPERATED EARS) IN RELATION TO AGE IN OSTEOGENESIS IMPERFECTA TYPE I

ANTON J. T. M. GARRETSEN, MD, PhD

COR W. R. J. CREMERS, MD, PhD

PATRICK L. M. HUYGEN, PhD

NUMEGEN, THE NETHERLANDS

Hearing loss was studied in relation to age in nonoperated ears in a group of 142 subjects with autosomal dominant osteogenesis imperfecta type I, which was compared to that in a random subsample of 70 subjects. In the $n = 142$ group, particularly below the age of 30 years, considerable selection (ie, for ear surgery) had occurred on hearing loss. The hearing threshold increased gradually with age. A hearing loss of greater than 30 dB (Fletcher index) was observed for 51% of the subjects older than 20 years and younger than 60 years. The median hearing loss progressed from the 10th to the 45th years of life with an average annual threshold increase (ATI) of 1 dB/y (0.5 to 4 kHz) up to 1.7 dB/y (8 kHz). Sensorineural loss accounted for 0.6 dB/y ATI at 0.5 to 4 kHz and 1.3 dB/y ATI at 8 kHz; conductive loss accounted for 0.4 dB/y ATI at all frequencies.

KEY WORDS — air-bone gap, air conduction, autosomal dominance, bone conduction, sensorineural hearing loss, socially adequate hearing.

INTRODUCTION

Hearing loss has been repeatedly reported as a major symptom of osteogenesis imperfecta (OI).¹⁻⁸ A variety of definitions of hearing loss and examination methods can be found in these reports.^{5,7-12} The groups of affected subjects examined do not usually represent random samples.

Despite the frequently described progression of hearing loss with age, age has seldom been taken into account in the analyses of hearing loss caused by OI. Obviously, the progression and the extent of hearing loss depend heavily on the age of the affected subjects. The type of hearing loss may be conductive, sensorineural, or mixed.

The present study analyzes the type and extent of hearing loss (in nonoperated ears only) in relation to age in a selected sample of 142 subjects with the autosomal dominant type I of OI.¹³ The results are compared to those of a previous study on a random sample of 70 affected subjects, who also had OI type I, from fully examined sibships.⁸

SUBJECTS AND METHODS

Subjects. We were able to collect a group of 142 subjects affected with OI type I for this study. This group is designated here "the selected sample," because either the patients had been previously elected for ear surgery or they volunteered for this study because of their hearing loss. The clinical diagnosis

had already been established elsewhere, based on the medical history and physical examination (ie, the presence of blue sclerae, fractures, hyperlaxity, and other possible characteristics in the proband and at least 2 generations of the family). Most of the patients were personally examined, except for 25 patients who had their clinical examination elsewhere at our request.⁸ Otorhinolaryngological examination had thus been performed in all of the subjects. Our previous reports concerned the findings and the results of ear surgery in subsamples of the present series.^{7,14,15} A segregation and penetrance analysis of hearing loss in a random subsample of 70 affected subjects had been established by fully examining the sibships in 30 families with at least 1 affected subject in 2 generations with elimination of the probands.⁸ Only 60 of these 70 patients had undergone clinical audiometry, 2 of them for 1 ear only. Of the other 10 patients, 2 were deceased, 6 were too young for audiometry, and 2 were living abroad.

Experimental Procedure. Pure tone thresholds in decibels hearing level (HL) were obtained from nonoperated ears under standard conditions in a sound-treated room at our clinic or elsewhere, according to ISO 8253¹⁶; calibration was performed according to ISO 389¹⁷ and ISO 7566.¹⁸ Subjects younger than 4 years of age were excluded from this study for practical reasons. Also excluded were subjects with hearing loss that could be attributed to causes other than OI, such as chronic otitis media. When several audio-

From the Department of Otolaryngology, University Hospital Nijmegen, Nijmegen, the Netherlands. This study was partly supported by grants from the Bio Children Rehabilitation Foundation, Arnhem, and the Junior Chamber, Apeldoorn, the Netherlands.

CORRESPONDENCE — Patrick L. M. Huygen, PhD, Dept of Otolaryngology, University Hospital Nijmegen, PO Box 9101, 6500 HB, Nijmegen, the Netherlands.

TABLE 1. SURVEY OF STUDIES ON HEARING LOSS IN OI

Authors	Year	No. of Pts	Limited to OI Type I	Definition of Hearing Loss*	Clinical Audiometry	Hearing Loss Related to Age	Random Sample	Proportion of Patients With Hearing Loss	
								No.	%
Bell ¹⁹	1928	346	—	—	—	—	—	228/346	66
Stoller ¹	1962	26	+	—	Unknown	—	1 family	11/26	42
Carey et al ²	1968	23	+	—	Unknown	—	1 family	10/23	43
Dufour and Bertrand ²⁰	1972	11	+	—	Unknown	—	1 family	4/11	36
Carruth et al ²¹	1978	10	+	—	+	+	— (3 families)	5/10	50
Quisling et al ⁴	1979	68	+	—	+ and portable	—	— (1 family)	20/42	48
Riedner et al ⁹	1980	70	—	+	+ and portable	+	— (13 families)	29/70	41
Cox and Simmons ¹⁰	1982	30	—	+	Only portable	+	— (5 families)	11/30	37
Shapiro et al ¹¹	1982	55	—	+	+	+	—	35/55	64
Pedersen ⁵	1984	173	—	+	+ and portable	+	— (75% of Danish population)	88/173	51
Stewart and O'Reilly ¹²	1989	56	—	+	+ and portable	+	—	31/53	58
Garretsen and Cremers ⁸	1991	70	+	+	+	+	+	30/70	43
This study		142	+	+	+	+	—	111/142	78

OI — osteogenesis imperfecta, portable — portable equipment implies less accuracy than usual.

*Definitions of hearing loss may differ among studies. + — present or applicable; — — absent or not applicable.

grams were available that had been performed at different ages, we took the one obtained at the youngest age. We used the following working definitions for the various types of hearing loss, paralleling the definitions given by Shapiro et al¹¹ and Pedersen.⁵

1. Conductive loss was defined as an average air-bone gap for the frequencies 0.5, 1, and 2 kHz, or else an average air-bone gap at 4 and 8 kHz, that was over 15 dB, and a corresponding bone conduction threshold of less than 15 dB.

2. Sensorineural hearing loss (SNHL) was defined as an average air conduction threshold that was at least 15 dB at the frequencies indicated above; the corresponding air-bone gap was less than 15 dB.

3. A mixed loss was defined as an average air-bone gap of at least 15 dB at the frequencies indicated above; the corresponding bone conduction threshold was at least 15 dB.

The synopsis on hearing loss and age in Tables 1, 2, 4, 5, 8-12, 19-21 and 25, 8-12, 21 provides a comparison to the relevant literature. The different types of hearing loss are shown in two age classes (Table 3¹¹). Detailed cross-tables (hearing loss by age) were com-

posed for the present group of 142 subjects and the previously reported random subsample of 70 subjects belonging to the fully examined sibships who were not selected for their hearing loss,⁸ in order to make a direct comparison (Tables 4 and 5). Hearing loss was substantiated by comparison with the corresponding 95th percentile value for presbycusis.²²

The statistical test used was the χ^2 test for a 2×2 table, with a level of significance of $p = .05$ (χ^2 value 3.84). Scatter plots were prepared for the data on hearing loss and age (Figs 1-3); details about the type of threshold data and the analyses involved are given where relevant.

RESULTS

The proportion of affected subjects having hearing loss and the relevant data from the literature are presented in Table 1. The present study shows the highest percentage (78%) of hearing loss reported so far. The exact application of the definitions of hearing loss given by Pedersen⁵ and Shapiro et al¹¹ yielded 80% and 83% hearing loss, respectively, for the present series; this finding indicates that there are only slight differences between the definitions. The

TABLE 2. SURVEY OF STUDIES ON HEARING LOSS IN OI AS DEPENDENT ON AGE

Authors	Year	Defini- tion of Hearing Loss	Number of OI Pts	Mean Age (Range)	4-9 y		10-19 y		20-29 y		<30 y		30-39 y		40-49 y		50-59 y		≥60 y		≥30 y	
					No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Carruth et al ²¹	1978	—	10	44 (8-77)	0/1		0/1				0/2		1/3		0/1		1/1		3/3		5/8	62
Riedner et al ⁹	1980	+	70	?	0/10	0 3/8	38 6/19		32 9/37		24 2/9		22 4/8		50 7/9		78 7/7		100 20/33		61	
Cox and Simmons ¹⁰	1982	+	30	20 (4-67)	1/8	13 4/10	40 2/5		40 7/23		30 1/3		2/3				1/1		4/7		57	
*Shapiro et al ¹¹	1982	+	55	?					18/37	49									17/18		94	
†Pedersen ⁵	1984	+	173	?	5/23	22 10/36	28 18/31		58 33/90		37 15/29		52 12/21		57 8/12		67 20/21		95 55/83		66	
Stewart and O'Reilly ¹²	1989	+	53	?		2/13	15 6/14		43 8/27		30 5/7		71 11/12		92 7/7		100		23/26		88	
Garretsen and Cremers ⁸	1991	+	70	?	2/18	11 13/21	62 6/13		46 21/52		40 1/8		13 2/3		3/4		3/3		9/18		50	
This study		+	142	27 (4-87)	4/13	31 24/38	63 26/31		84 54/82		66 23/24		96 17/18		94 9/10		90 8/8		100 57/60		95	

Proportion of patients with hearing loss within given decade is presented. Percentages are given only for n > 4.

+ — present or applicable, — — absent or not applicable.

*Original classes 1st to 3rd decades and 4th to 7th decades.

†Estimated from illustrations in original paper.

random subsample reported by Garretsen and Cremers⁸ yielded a much lower percentage (43%) of hearing loss.

It is remarkable that the family studies^{1,2,4,9,10,20,21} yielded a similar low percentage (90/212 or 42%). The latter studies, including the work by Garretsen and Cremers,⁸ indicate a mean percentage of 43% for hearing loss, which has a corresponding proportion of 120 to 282. This differs significantly from the present and other nonfamily studies^{5,11,12,19} for which the percentage of hearing loss is 64%, with a corresponding proportion of 493 to 769.

Table 2 shows the relation between the proportion

TABLE 3. TYPES OF HEARING LOSS IN OI TYPE I (SELECTED SAMPLE)

	<30 y		≥30 y		Total	
	No.	%	No.	%	No.	%
Normal hearing	52	34	8	6	60	21
Conductive hearing loss	3	2	0	0	3	1
Mixed hearing loss	56	37	89	68	145	51
Sensorineural hearing loss	27	18	25	19	52	18
CHOI*	10	7	7	5	17	6
Deafness	3	2	2	2	5	2
Total	151	100	131	100	282†	100

Number of ears in 142 patients with OI type I with normal hearing or specified type of hearing loss in selected sample (282 ears) in total and in two age classes.

*Hearing loss "characteristic of OI" according to Shapiro et al.¹¹

†Threshold unknown in 1 ear in 2 cases.

of subjects with hearing loss among the affected subjects and the age of the subjects (in decades) for the present study and some other relevant studies.^{5,8-12,21} For those below 30 years of age, hearing loss was observed for 96 of 268, or 36%. The present study, however, had a significantly higher proportion of subjects with hearing loss (54/82 or 66%). For those age 30 years and over, the present study and the one by Shapiro et al¹¹ had higher proportions of hearing loss (57/60 or 95% and 17/18 or 94%, respectively) than the other selected groups of patients,^{5,12} which showed an average proportion of 78 to 109 (72%). The proportions corresponding with 94% and 95% are significantly (see Subjects and Methods) higher than those for all the family studies^{8-10,21} taken together, which yielded a proportion of subjects with a hearing loss of only 38 of 66 (58%). The data in Table 2 clearly show that the proportion of subjects with hearing loss increases with age. This is particularly clear in the larger series. An exception is the series published by Garretsen and Cremers,⁸ which showed large deviations from the general trend in the second and fourth decades. Together with the present series, of which it represents a subsample, the series by Garretsen and Cremers differs significantly from the others in the high proportion of hearing loss in affected subjects in their second decade of life.

The statistics for normal hearing and the various types of hearing loss are presented in Table 3,¹¹ including two age classes. Normal hearing was most frequent at a younger age. Conductive loss was found only in a few young affected subjects. Mixed hearing

TABLE 4. PROPORTION OF HEARING LOSS WITHIN GIVEN THRESHOLD RANGE BY DECADE IN OI TYPE I (SELECTED SAMPLE)

Hearing Loss (dB)	4-9 y		10-19 y		20-29 y		30-39 y		40-49 y		50-59 y		≥60 y	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0-10	21/26	81	36/76	47	16/60	27	6/50	12	10/36	28	5/20	25		
15-30	5/26	19	14/76	18	14/60	23	12/50	24	9/36	25	8/20	40	3/16	19
35-50			16/76	21	21/60	35	12/50	24	5/36	14	1/20	5	7/16	44
55-90			8/76	11	6/60	10	17/50	34	9/36	25	3/20	15	5/16	31
95			1/76	1	3/60	5	2/50	4	3/36	8	3/20	15	1/16	6
>30	0/26	0	25/76	33	30/60	50	31/50	62	17/36	47	7/20	35	13/16	81

Hearing loss in decibels hearing level (Fletcher index). Shading indicates median hearing loss for each decade.

loss was the most frequent type of hearing loss, and the frequency increased significantly with age. Constant proportions, independent of age, were found for SNHL; for the type of SNHL occurring predominantly or exclusively at 8 kHz, which was considered by Shapiro et al to be "characteristic of OI"^{11(p2122)} (CHOI); and for deafness.

Table 4 presents the proportions of patients with a given hearing loss by age (in decades) for the present series of selected subjects. Table 5 is a similar table for the random subsample.⁸ In Tables 4 and 5, the median hearing loss per decade is also presented. In the present selected sample, the median hearing loss increased from the first decade, at 0 to 10 dB, up to about 30 to 35 dB in the third decade (Table 4). In the random subsample (Table 5), the median hearing loss only increased after the fourth decade. In the selected sample from the 20th year of life, the air conduction threshold was higher than 30 dB for 51% (85/166) of the affected subjects 20 to 59 years of age (Table 4); a further (significant) increase in this proportion could only be noted in the seventh decade. Testing the proportions in the cells for 0 to 10 dB and the second or third decade in the random sample (Table 5) against the corresponding proportions in the selected sample (Table 4) showed that the random sample had

significantly higher proportions in these cells (58% as compared to 47%, and 70% compared to 27%, respectively; see Tables 4 and 5). The difference in the relation between the air conduction threshold and age between the two series (random and selected cases) appeared to be significant. With increasing age, the two series tended to develop a similar (median) hearing loss, but the series of selected cases showed selection, especially at a younger age. For each pair of corresponding cells in Tables 4 and 5, we calculated the difference in proportion between the random and the selected series. In this way we obtained a difference distribution that showed a higher proportion than the random sample (Table 5) in the following cells: 35 to 50 dB for 10 to 19 years (9/24 or 38%); 55 to 90 dB for 10 to 19 years (6/24 or 25%); and 35 to 50 dB for 20 to 29 years (19/40 or 48%). For the three cells combined, the distribution of the difference indicated a proportion of 34/64 (53%), while this combination for the random sample (Table 5) yielded a proportion of only 11/72 (15%); the difference is significant. The difference distribution (not shown) had a median threshold for all age classes beyond the first decade that coincided almost invariably with the class 35 to 50 dB.

In order to establish further characteristics of the

TABLE 5. PROPORTION OF HEARING LOSS WITHIN GIVEN THRESHOLD RANGE BY DECADE IN OI TYPE I (RANDOM SAMPLE)

Hearing Loss (dB)	4-9 y		10-19 y		20-29 y		30-39 y		40-49 y		50-59 y		≥60 y	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0-10	20/24	83	30/52	58	14/20	70	2/2		2/6	33	2/8	25		
15-30	4/24	17	12/52	23	4/20	20			4/6	67	5/8	63	1/6	17
35-50			7/52	13	2/20	10							4/6	67
55-90			2/52	4							1/8	13	1/6	17
95			1/52	2										

Hearing loss in decibels hearing level (Fletcher index). Percentages are only for n > 4. Shading indicates median hearing loss for each decade.

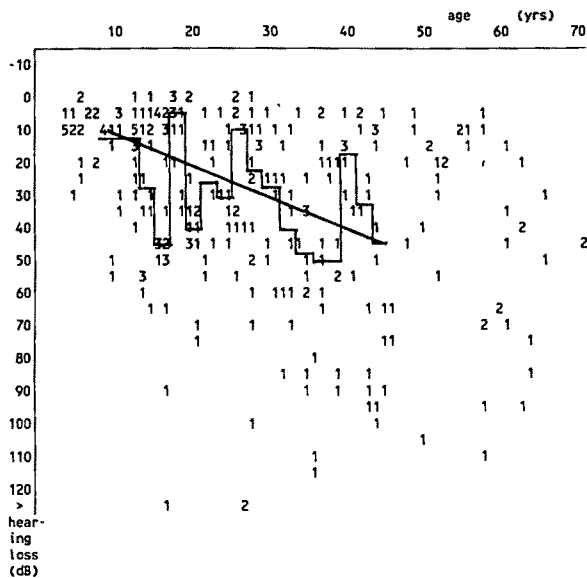


Fig 1. Mean air conduction threshold (decibels hearing level [dB HL]) for 0.5, 1, and 2 kHz (ie, Fletcher index) in selected group (282 ears, without or before surgery) plotted against age (in years). Numbers in Figure ($n < 10$) correspond with number of ears with given combination of certain hearing loss and age at appropriate coordinates. Histogram-like contour represents interconnected median values for hearing for age classes (width 2 years) covering 10 to 45 years of age (10-11, 12-13, ..., 42-43, 44-45 years). Linear trend is shown from 10 dB at 10 years toward 45 dB at 45 years (continuous sloped line) as crude approximation to development of this "biennial" median hearing loss with age.

hearing loss as it develops with age, we prepared separate scatter plots for the present series, for air conduction, bone conduction, and air-bone gap at various frequencies. Those for the mean hearing loss at 0.5, 1, and 2 kHz are depicted in Figs 1-3. In the plots we have indicated the median hearing loss (class width 2 years in order to obtain $n > 4$ for a reliable estimation of the median). It can be seen from Figs 1-3 that we utilized the median values to estimate a linear trend of hearing loss with increasing age (continuous sloped line). It should be noted that this "trend analysis" was limited to the age range 10 to 45 years for the following reasons.

1. In the age groups younger than 10 years and older than 45 years of age, there were relatively few data available, especially regarding bone conduction and air-bone gap; ie, a class width of over 2 years would be required in order to obtain $n > 4$.

2. This particular age range showed the most prominent increase in hearing loss.

3. The distributions of the air and bone conduction thresholds at 4 and 8 kHz, particularly in the older patients, appeared to be bimodal, analogous to those of otosclerosis²³; this bimodality would complicate a meaningful analysis.

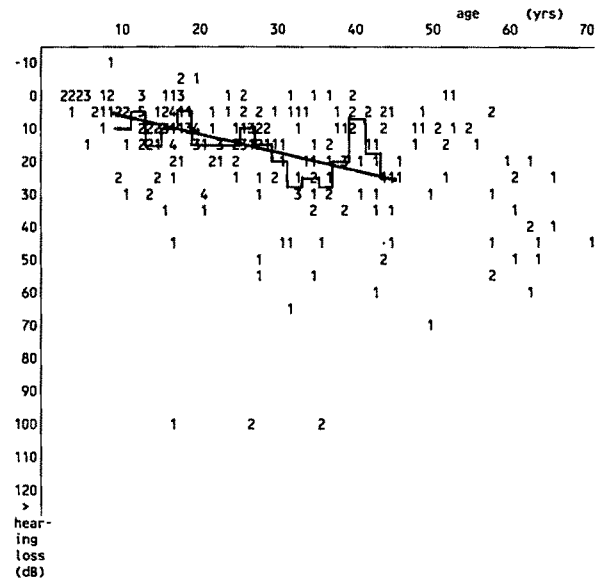


Fig 2. Mean bone conduction threshold (dB HL) for 0.5, 1, and 2 kHz in selected group (282 ears, without or before surgery) plotted against age (in years). See Fig 1 for legend. Median threshold trend is shown from 5 dB at 10 years toward 25 dB at 45 years.

The results of the analysis are presented in Table 6. This Table also presents the results of a similar analysis, restricted to air conduction, in the random sample. The analysis of Table 6 yields the following results for the selected group.

1. The SNHL increased with both age and frequency. It should be noted (Fig 1) that the hearing loss (ie, air conduction) exceeded the loss that can be reasonably attributed to presbycusis, for which the mean 95th percentile threshold corresponding to the Fletcher index is about 11 dB at 20 years of age and increases through 17 dB at 45 years to about 33 dB at 70 years according to ISO 7029²²; the 95th percentile of presbycusis appeared to coincide with the 25th percentile (averaged over 20 to 45 years) of our patient group.

2. The conductive component increased with age, to a similar degree for all frequencies.

3. The major increase in hearing loss was invariably shown by the SNHL: 0.6 dB/y for the mean of 0.5, 1, and 2 kHz; 0.7 dB/y at 4 kHz; and 1.3 dB/y at 8 kHz. The increase in air-bone gap with age was limited to about 0.4 dB/y.

DISCUSSION

The present report on a group of 142 selected subjects with OI type I shows the highest proportion of hearing loss reported so far. A comparison with the random sample⁸ that formed part of this series showed that considerable selection on hearing loss had taken place at a rather young age. It is interesting

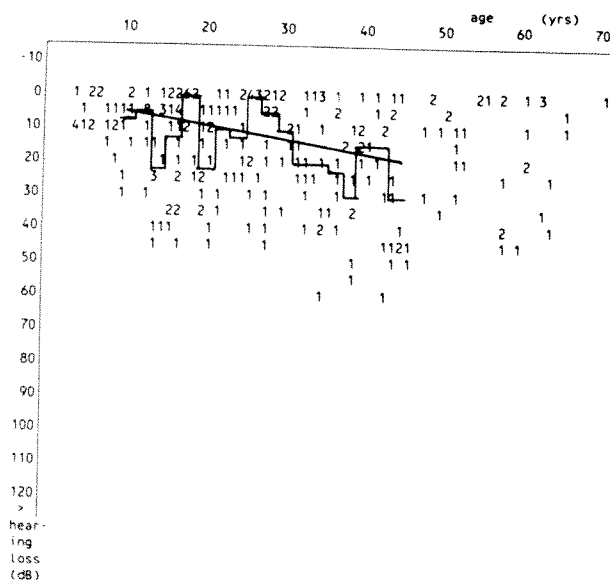


Fig 3. Mean air-bone gap (dB HL) for 0.5, 1, and 2 kHz in selected group (282 ears, without or before surgery) plotted against age (in years). See Fig 1 for legend. Median threshold trend is shown from 5 dB at 10 years toward 20 dB at 45 years.

to see that the median of the distribution of the difference in the proportion of hearing loss between the selected and the random samples — which relates to the group of affected subjects who were selected for ear surgery because of their hearing loss — coincides with the class of 30- to 50-dB loss in air conduction threshold at any age from 10 years onward. Apparently, the presumed selection has taken into account that a Fletcher index (in the nonoperated, presumably the best, ear) of more than 30 dB renders the hearing capacity less adequate socially.

The findings relating to Table 1 suggest the possibility that the family studies involved series of patients that are more similar to a random sample than to a selected sample. Therefore, it seems justified to consider the prevalence of hearing loss in OI type I as

being 43%. It appears from Table 2 that the series with selected patients distinguish themselves especially by a greater hearing loss at an increasing age (30 years and older). In the present series, the proportion of subjects with hearing loss was higher than in any other series published so far, and the hearing loss occurred also at a younger age. It may be relevant that self-selection of affected subjects with hearing loss played a role, because a patient association lent its active support to the present study.

When comparing the present selected sample (Table 3) to other selected samples as closely as possible, as regards the type of hearing loss, we see that the present series included more cases of mixed hearing loss, especially in the older patients, than the series published by Shapiro et al¹¹ and Pedersen.⁵ The present series also showed considerably more SNHL (only) at a young age as compared to those series. This only holds true as regards the comparison with Shapiro et al if CHOI is set apart. This type of hearing loss, which was found by these authors in both the lower and higher age groups in 47% of the ears, was only found in a minor percentage in the present series (Table 3), as well as in Pedersen's series. Others have also found that the predominant type of hearing impairment in OI was the mixed type, although to a much lesser degree than in the present study.^{9,10,12,21} Riedner et al⁹ and Stewart and O'Reilly¹² indicated that conductive loss, as far as it was present, prevailed in the younger age group. Stewart and O'Reilly, just as we did, described an age-independent proportion for SNHL.

The present series showed gradual progression in hearing loss, ie, significantly more than could be explained on the basis of presbycusis, with age: only 25% of our patients within the age range of 20 to 45 years had a Fletcher index equal to or below that derived for the 95th percentile of presbycusis. Given the large numbers of observations involved, there is

TABLE 6. MEDIAN HEARING LOSS AND ANNUAL THRESHOLD INCREASE IN OI TYPE I

		Median Hearing Loss (dB HL)		Annual Increase (dB/y)
		10 y	45 y	
0.5, 1, 2 kHz	Bone conduction	5	25	20/35 = 0.6
	Air-bone gap	5	20	15/35 = 0.4
	Air conduction	10 (10)	45 (10)	35/35 = 1 (0)
4 kHz	Bone conduction	5	30	25/35 = 0.7
	Air-bone gap	5	20	15/35 = 0.4
	Air conduction	10 (10)	50 (20)	40/35 = 1.1 (10/35 = 0.3)
8 kHz	Bone conduction	5	50	45/35 = 1.3
	Air-bone gap	5	20	15/35 = 0.4
	Air conduction	10 (10)	70 (30)	60/35 = 1.7 (20/35 = 0.6)

Median hearing loss (decibels hearing level) for 142 selected patients with OI type I at ages of 10 and 45 years (estimated from linear trend), for mean at 0.5, 1, and 2 kHz, for 4 kHz, and for 8 kHz, in terms of air conduction, bone conduction, and air-bone gap, together with annual increase in threshold (decibels per year). Figures for random subsample of 70 patients with OI type I are in parentheses.

no doubt that the difference is significant. A considerable increase in hearing loss took place especially from the 10th to the 45th years of life, which comprised a considerable increase in SNHL and only a limited increase in conductive loss (Table 6). We studied individual follow-up data covering 2 through 24 years (average 9.6 years) on all subjects who had ear surgery.¹⁵ Between 1 year and the average 9.6 years after surgery, the mean hearing level in the operated ear dropped by 6.5 dB. This almost equals the indication given in Table 6, despite the fact that the conductive component in postoperative hearing loss plays a lesser role. Also, the nonoperated ear showed a similar progression in hearing loss.¹⁵

It was intriguing to see that the increase in air-bone gap seemed to be limited to about 0.4 dB/y, independent of the frequency.

Less than 50% of the affected subjects older than 20 years of age had socially adequate hearing (ie, average of 30 dB or better). From a previous report,¹⁵ it appears that among 58 operated ears, there was only 1 ear with socially adequate hearing preoperatively. Postoperatively, 24 ears (41%) did not reach the level of socially adequate hearing.

The above-stipulated progression of hearing loss

with age (here evaluated in nonoperated ears only) occurs in both the nonoperated and the operated ears, and to a similar degree.^{7,15} The average hearing gain immediately after the operation is about 24 dB¹⁵; thus, given a progression in hearing loss such as presented in Table 6, the hearing gain tends to disappear after a follow-up interval of several decades, as, indeed, seemed to be the case in the patients with a long-enough follow-up.

From an earlier study, it is clear that a relatively greater preoperative SNHL was present in the patients who were operated on at a young age.⁷ It is possible that in this group of patients, the progression of SNHL that occurs preoperatively and postoperatively is greater than in the average patient. Stapes surgery is not recommended in cases of considerable hearing loss if the conductive component is too small.

Given the present findings on the natural history of hearing loss in OI, combined with our previously reported findings on the results of surgery, it seems justified to inform — if necessary — any young affected subjects whose profession depends heavily on auditory communication that they may have to cope with increasing hearing problems at a more advanced age.

ACKNOWLEDGMENTS — We thank the patients and their relatives for their cooperation, the Dutch otolaryngological surgeons who helped to collect the data and examine patients, and the Dutch Association of Patients With Osteogenesis Imperfecta for contacting some of the patients.

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NARROWEST (ISTHMUS) PORTION OF EUSTACHIAN TUBE: A COMPUTER-AIDED THREE-DIMENSIONAL RECONSTRUCTION AND MEASUREMENT STUDY

MASAHARU SUDO, MD
AKIHIRO IKUI, MD, DMSc

ISAMU SANDO, MD, DMSc
CHIAKI SUZUKI, MD, DMSc

PITTSBURGH, PENNSYLVANIA

Nine normal human temporal bones from persons 16 to 88 years old were studied by computer aided three-dimensional reconstruction and measurement. The length of the eustachian tube (ET) lumen in three portions (from pharyngeal orifice to tympanic orifice: cartilaginous, junctional, and bony) averaged 23.6 ± 4.3 mm, 3.0 ± 1.9 mm, and 6.4 ± 2.6 mm. The narrowest portion of the ET lumen was in the cartilaginous portion in all cases: 20.5 ± 4.2 mm from the pharyngeal orifice and 3.1 ± 1.6 mm from the pharyngeal margin of the junctional portion. The cross-sectional area of the narrowest portion was 0.65 ± 0.2 mm². The tendon of the tensor veli palatini muscle (TVPM) inserted into the lateral lamina in the narrowest portion of the ET lumen in five of nine cases. These results suggest that contraction of the TVPM opens the narrowest portion of the ET lumen to ventilate the middle ear and that this portion also plays a role in protecting the middle ear.

KEY WORDS — computer-aided three-dimensional reconstruction, eustachian tube cartilage, eustachian tube isthmus, eustachian tube lumen, tensor veli palatini muscle.

INTRODUCTION

The eustachian tube (ET) has three functions: ventilation, protection, and clearance of the middle ear (ME).¹ For ventilation, the ET lumen is opened actively²⁻⁵ by constriction of the tensor veli palatini muscle (TVPM), which inserts along the cartilaginous portion of the ET. The narrow portion of the lumen protects the ME by preventing foreign bodies, secretions, and bacteria from entering this space. It has traditionally been thought that the narrowest portion of the ET coincides with the junctional or isthmus region, which is located between the cartilaginous and bony portions of the ET.⁶⁻⁹ Other investigators¹⁰⁻¹² speculated that the narrowest portion of the ET is in the cartilaginous portion. Such a location would allow the ET to open effectively for ventilation, because it has a broad area of TVPM inserting into the well-developed lateral lamina of the ET cartilage. Zöllner¹⁰ used the term "physiological isthmus" for this portion of the ET.

Recently, Sadé et al^{11,12} studied the histologic features of six portions of the ET: the pharyngeal portion, cartilaginous portion, near-isthmus, isthmus, post-isthmus, and pretympa. They measured the cross-sectional area of each of these portions and found the near-isthmus portion to be the narrowest. However, measurements made on histologic sections

may be inaccurate, because it is extremely difficult to cut sections exactly perpendicular to the ET axis. In our experience, the typical variation of 10° to 20° adds significant error to these determinations. To overcome this problem, this study has used computer-aided three-dimensional (3-D) reconstruction and measurement methods to measure the true cross-sectional area of the ET.

Further, to avoid confusion arising from variable definitions of the term "isthmus," we labeled the portion of the ET between the cartilaginous and bony portions the "junctional portion." The exact location of this portion of the ET is hard to determine, because the cartilage and bone overlap. Our reconstruction and measurement methods provide an objective means to identify the location of the junctional portion.

METHODS

The materials for this study were nine temporal bone-ET specimens obtained from nine individuals, 16 to 88 years of age at death. None of the individuals had any known congenital anomaly or a history of ear disease. The specimens were fixed in 10% formalin, decalcified in 5% trichloroacetic acid, dehydrated in graded concentrations of ethanol, and embedded in celloidin.¹³ After reference marks had been made in the blocks for 3-D reconstruction, the blocks were

From the Elizabeth McCullough Knowles Otopathology Laboratory, Division of Otopathology, Department of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania. This study was supported by Research Grant 5 R01 DC 00123 from the National Institutes of Health/National Institute on Deafness and Other Communication Disorders.

CORRESPONDENCE — Isamu Sando, MD, DMSc, Division of Otopathology, Dept of Otolaryngology, University of Pittsburgh School of Medicine, Suite 153, Eye & Ear Institute Building, 203 Lothrop St, Pittsburgh, PA 15213.

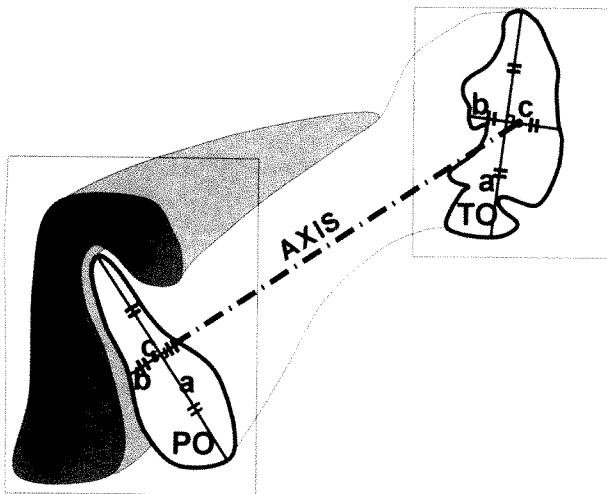


Fig 1. Schematic drawing along long axis of left eustachian tube (ET) viewed from anteriorly. **AXIS** — axis of ET, **C** — ET cartilage, **PO** — pharyngeal orifice, **TO** — tympanic orifice, **a** — long axis of cross section of ET, **b** — line perpendicular to "a" and crossing "a" in middle, **c** — midpoint of line "b" representing center point of pharyngeal or tympanic orifice.

sectioned vertically at 30 μ m. Every 20th section in the cartilaginous portion near the junctional portion, the junctional portion, and the bony portion, and every 40th section for the remainder of the temporal bone, were stained with hematoxylin and eosin and prepared for light microscopic study. These histology slides were then used for reconstruction. The equipment used for reconstruction consisted of a personal computer (NEC PC-9801RL), a frame buffer (Sapience HyperFrame +) for full-color images, a digitizer (Hitachi HDG-1515, resolution 0.1 mm), and a printer. The software developed by Takagi and Sando¹⁴ and modified by Fujita and Sando¹⁵ was revised by the first author (M.S.) so that accurate measurements could be made of true cross-sectional

areas ("virtual cuts") of the ET in 3-D fashion.

For each specimen, the ET lumen, the ET cartilage, the insertion of the TVPM tendon into the lateral lamina of the cartilage, and the temporal and sphenoid bones bordering the ET were reconstructed. First, the pharyngeal orifice of the ET was defined as the area at the most anterior end of the torus tubarius (posterior lip portion), and the tympanic orifice of the ET was defined as the area at the anterior margin of the tympanic annulus. Second, the center of each orifice was determined (Fig 1). Third, the axis of the ET was determined by connecting the centers of the pharyngeal and tympanic orifices. These landmarks were used to create 200 computer images of cross sections perpendicular to the axis of the ET with an error of less than 0.01°. The cross-sectional areas of the ET lumen, ET cartilage, ET bone, and TVPM were then measured from these 200 "virtual cuts." The computer software produced, semiautomatically, a file containing data prints for the cross-sectional images; the data file was then read into a spreadsheet software program for further analysis and graphing. We limited measurement of the cross-sectional area to the bony margins that were found within 0.2 mm of the lumen (Fig 2).

The pharyngeal margin of the junctional portion was defined as the point where the most anterior margin of bone surrounding the ET lumen was seen. The tympanic margin of the junctional portion was defined as the point where the most posterior margin of cartilage was seen. Finally, we graphed each of the three parts of the ET (the cartilaginous portion, the junctional portion, and the bony portion) and measured their lengths, as well as the range of the insertion site of the TVPM into the cartilage.

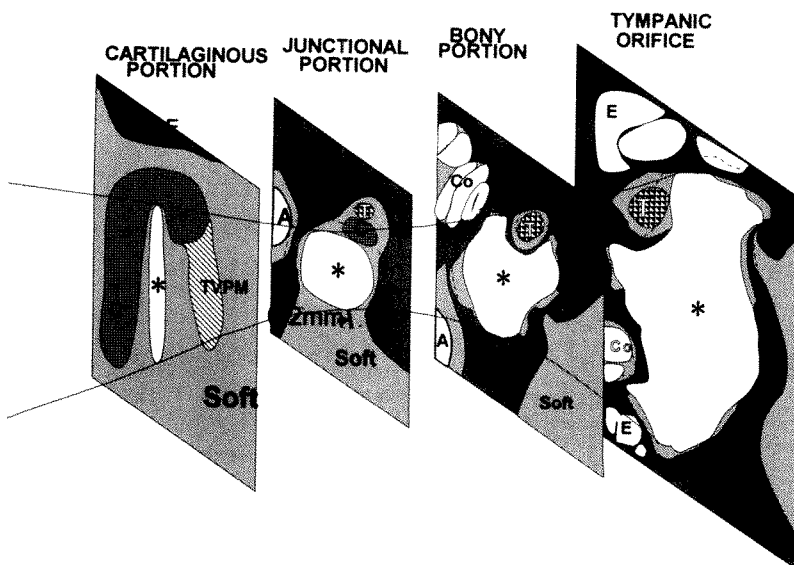


Fig 2. Schematic drawings of ET lumen (*) and its surrounding structures in vertically cut cross sections at four representative points along long axis of ET. Dashed lines are 0.2 mm from margin of ET lumen. **A** — internal carotid artery, **BONE** — bony tissue, **C** — ET cartilage, **Co** — cochlea, **D** — bony tissue within 0.2 mm of ET lumen, **E** — air cells, **Soft** — connective tissue, **T** — tensor tympani muscle, **TVPM** — tensor veli palatini muscle.

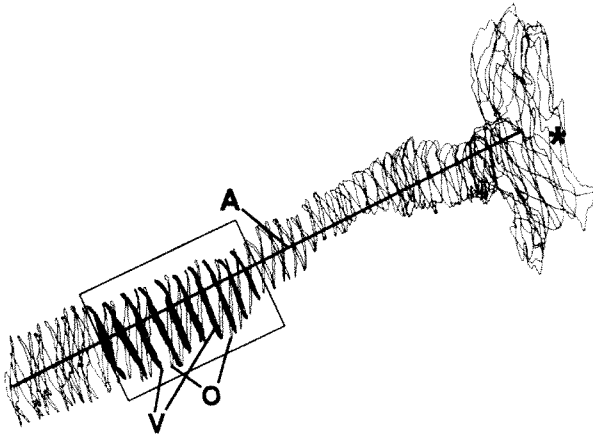


Fig 3. Wire frame model of ET lumen of 28-year-old woman. Lines that are oblique to longitudinal axis (A) of ET (O) represent plane of histologic sections. Lines that are perpendicular to ET axis (V, shaded in inset) represent "virtual cuts" that were used to measure cross-sectional area. * — tympanic cavity.

RESULTS

Figure 3 shows a "wire frame" reconstruction of the ET lumen as seen along its long axis. The total length of the ET in this specimen (from a 28-year-old woman) was 34.17 mm. Figure 4 shows graphs of cross-sectional areas of the ET structures in the same specimen illustrated in Fig 3. In this specimen, the lengths of the cartilaginous, junctional, and bony portions were 22.63 mm, 0.84 mm, and 10.07 mm. The cross-sectional area of the lumen was 7.79 mm² at the pharyngeal orifice, 0.67 mm² at the narrowest portion (which was within the cartilaginous portion), 0.86 mm² at the pharyngeal margin of the junctional portion, 1.21 mm² at the tympanic margin of the junctional portion, and 18.1 mm² at the tympanic orifice of the bony portion. The narrowest portion

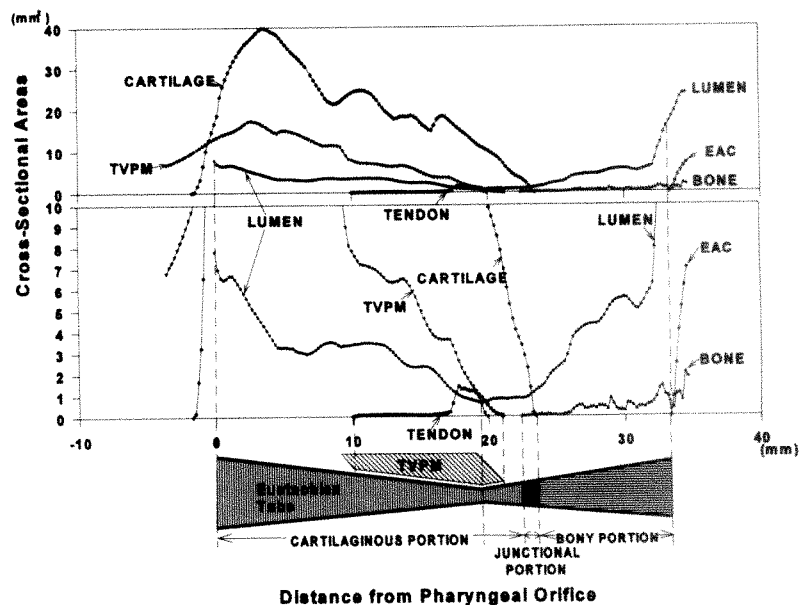
was within the cartilaginous portion, 20 mm from the pharyngeal orifice, in the region where the TVPM inserted into the lateral lamina of the ET cartilage.

The averages and standard deviations for the true cross-sectional areas, along the ET, of the lumen, TVPM, cartilage, and bone of the nine cases are shown in Fig 5. The cross-sectional areas of the TVPM and the cartilage varied considerably among specimens. The areas of the pharyngeal and the tympanic orifices of the lumen varied as well. However, the area of the lumen adjacent to the narrowest portion showed remarkably less variation. The cross-sectional areas of the pharyngeal orifice (7.37 ± 5.65 mm²), the narrowest portion (0.65 ± 0.19 mm²), the pharyngeal margin (1.69 ± 0.92 mm²) and the tympanic margin (3.35 ± 2.04 mm²) of the junctional portion, and the tympanic orifice (18.63 ± 5.48 mm²) of each specimen are shown in Table 1.

The averages and standard deviations for these cross-sectional areas were plotted and are shown in Fig 6. As Fig 6 shows, the narrowest portion of the lumen is within the cartilaginous portion of the ET and also within the area for insertion of the TVPM.

The lengths of the cartilaginous, junctional, and bony portions of the ET in the nine specimens are shown in Fig 7 and Table 2, which also show the location of the narrowest portion of the lumen and the area for the insertion of the TVPM into the lateral lamina. In every case, the location of the narrowest portion of the lumen was within the cartilaginous portion. In five of the nine specimens, the narrowest point also lay within the area for insertion of the TVPM into the ET cartilage. As Fig 7 shows, in no case did the TVPM insert into the junctional portion

Fig 4. Two graphs shown in upper portion demonstrate cross-sectional areas, in different magnifications, of ET lumen, cartilage, TVPM, its tendon, bone within 0.2 mm of ET lumen, and external auditory canal (EAC) from same specimen illustrated in Fig 3. Schematic drawing in lower portion shows location of insertion of TVPM into ET cartilage and cartilaginous, junctional, and bony portions of ET as they relate to actual ET graphed above.



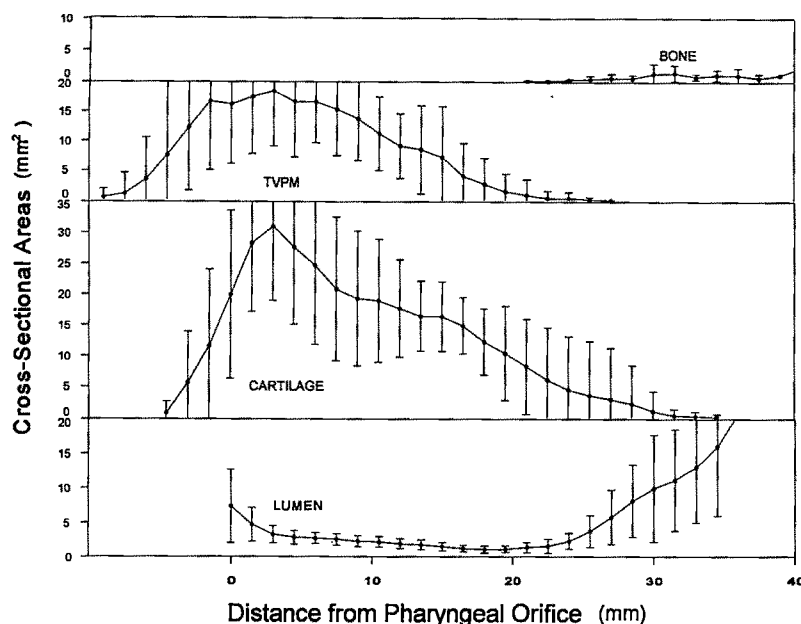


Fig 5. Plots of averages and standard deviations (bars) of true cross-sectional areas of bone within 0.2 mm from ET lumen, TVPM, ET cartilage, and ET lumen.

of the cartilage.

Table 2 shows the lengths of each part of the ET, which averaged 23.57 ± 4.26 mm for the cartilaginous portion, 3.04 ± 1.90 mm for the junctional portion, and 6.36 ± 2.61 mm for the bony portion. The location of the narrowest portion of the lumen was 20.52 ± 4.22 mm from the pharyngeal orifice. Table 2 also shows the range for insertion of the TVPM into the lateral lamina of the cartilage. The width of the area of insertion was 8.27 ± 2.54 mm, with the edges located 12.62 ± 4.87 mm and 20.89 ± 2.78 mm from the pharyngeal orifice.

DISCUSSION

The location of the narrowest portion of the ET has very important implications regarding its proposed role in actively ventilating the ME. However, quantitative information has been unavailable previously,

as a consequence of both the preconception that the isthmus is the narrowest portion of the ET, and the difficulty of sectioning specimens exactly perpendicular to the axis of the ET to permit measurement of true cross sections. Reconstruction techniques, though, enabled us to generate computer images of true cross sections of the ET and to measure them accurately. The results showed that the narrowest portion of the ET is in the cartilaginous portion, supporting both the reports by Zöllner¹⁰ and Sadé et al^{11,12} and the hypothesis that the narrowest portion is involved in the ET's active ventilatory function.²⁻⁵ Furthermore, in five out of nine cases, the area for insertion of the TVPM into the ET cartilage overlapped the narrowest portion. This finding is consistent with the hypothesis that the narrowest portion of the ET serves to protect as well as ventilate the middle ear.

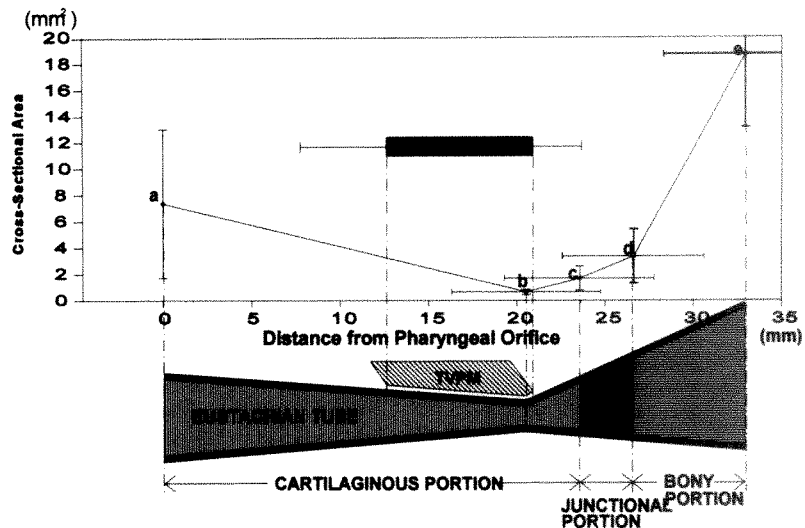
If, as just hypothesized, the narrowest portion of

TABLE 1. CROSS-SECTIONAL AREAS OF EUSTACHIAN TUBE

Case	Pharyngeal Orifice	Narrowest Portion	Junctional Portion		
			Pharyngeal Margin	Tympanic Margin	Tympanic Orifice
1	3.95	0.72	1.99	6.11	18.76
2	4.76	0.58	2.84	3.11	25.56
3	7.79	0.67	0.86	1.22	18.13
4	5.97	0.39	1.18	2.23	16.23
5	21.32	0.48	0.55	1.47	15.66
6	3.50	1.00	2.56	3.37	10.33
7	4.75	0.73	0.98	7.14	14.30
8	4.11	0.77	1.28	1.94	27.85
9	10.16	0.48	2.97	3.56	20.86
Average	7.37	0.65	1.69	3.35	18.63
SD	5.65	0.19	0.92	2.04	5.48

Data are in square millimeters.

Fig 6. Averages and standard deviations of cross-sectional areas (y-axis) and locations (x-axis) of ET at pharyngeal orifice (a), narrowest portion (b), pharyngeal (c) and tympanic (d) margins of junctional portion, and tympanic orifice (e) are plotted. Vertical and horizontal bars indicate standard deviations. Averages and standard deviations for tympanic and pharyngeal margins of insertion of TVPM are also plotted (black bar). Schematic drawing of "average" ET is shown below graph.



the ET can be opened actively by constriction of the TVPM and the junctional portion cannot, then the posterior limit of active opening of the ET might logically be the pharyngeal margin of the junctional region. As Table 1 shows, the average cross-sectional area of the narrowest portion of the ET is 0.65 mm^2 . On active opening, the ET might be expected to have a cross section of 1.69 mm^2 , which is the average cross-sectional area of the pharyngeal margin of the junctional portion.

The cross-sectional area of the ET varied more at the pharyngeal orifice and the tympanic orifice than

at other points along the ET (Figs 5 and 7), particularly the narrowest portion, which was remarkably consistent in cross-sectional area. The relative uniformity of the area across the lumen in the narrowest part is compatible with the hypothesis that the narrowest portion plays an important role in protecting the middle ear.

Our study complements that of Sadler-Kimes et al,¹⁶ who reported using computer-aided reconstruction to determine the average cross-sectional areas of the ET lumens in specimens obtained from 13 children. In this present study, we measured the cross section of the ET lumen throughout its course so that we could identify the narrowest portion.

We introduced the term "junctional portion" to refer to the area often called the "isthmus portion" of the ET, where tissue around the lumen changes from mostly cartilage to mostly bone. We thus refer to the three portions of the ET as the cartilaginous, junctional, and bony portions. We further suggest referring to the "narrowest portion" rather than the "isthmus portion" to distinguish the physiologically important point of smallest cross-sectional area, which we found in this study to be located in the cartilaginous portion of the ET. Also in this study, we determined the length of the junctional portion ($3.04 \pm 1.90 \text{ mm}$) for the first time.

Finally, any study that involves histologic processing of specimens may produce artifacts such as shrinkage of the section (which has been estimated at 10%¹⁷ for the process used in this study). Thus, our results are no more inaccurate because of such artifacts than results of others using similar histologic processing techniques. However, our computer-aided 3-D reconstruction and measurement method is more reliable and accurate than other techniques currently in use for investigating the 3-D anatomy of the ET.

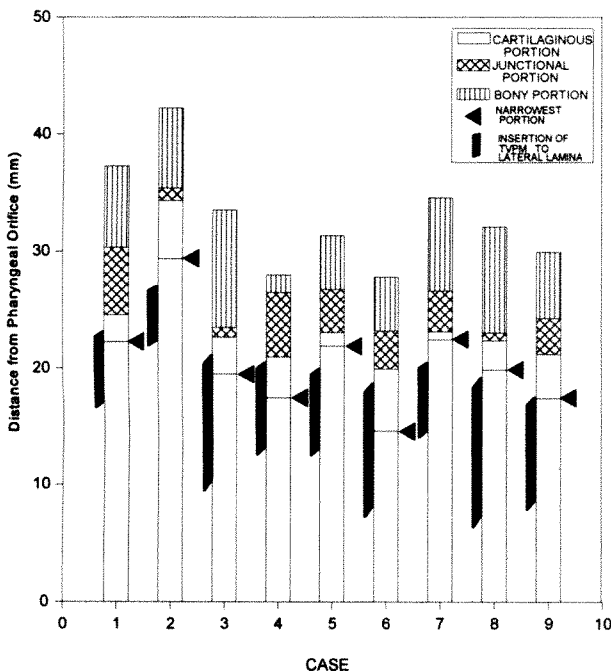


Fig 7. Lengths of cartilaginous, junctional, and bony portions of each of nine ETs are plotted. Location of narrowest portion of each ET and range of insertion of TVPM into cartilage are also shown. Note that in all specimens, narrowest portion is in cartilaginous portion.

TABLE 2. LENGTHS AND LOCATIONS OF EUSTACHIAN TUBE SEGMENTS

Case	Age (y)	Sex	Length			Location			Length
			Cartilaginous Portion	Junctional Portion	Bony Portion	Narrowest Point*	Insertion of TVPM to Cartilage		Range
							Pharyngeal Margin*	Tympanic Margin*	
1	17	F	24.55	5.78	6.95	22.23	16.94	23.16	6.22
2	70	M	34.34	1.05	6.82	29.36	22.31	27.00	4.69
3	28	F	22.63	0.84	10.07	19.48	10.16	21.16	11.00
4	16	M	20.97	5.51	1.49	17.47	12.99	20.53	7.54
5	61	F	23.05	3.70	4.60	21.90	12.94	20.05	7.11
6	38	F	19.92	3.25	4.63	14.59	7.97	18.76	10.79
7	71	F	23.12	3.51	7.94	22.42	14.53	20.54	6.01
8	70	M	22.36	0.66	9.07	19.84	7.30	19.21	11.91
9	88	F	21.21	3.06	5.66	17.40	8.47	17.59	9.12
Average	51.00		23.57	3.04	6.36	20.52	12.62	20.89	8.27
SD	26.63		4.26	1.90	2.61	4.22	4.87	2.78	2.54

Data are in millimeters.

TVPM — tensor veli palatini muscle.

*Points are represented by distance in millimeters from pharyngeal orifice of eustachian tube.

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RECURRENT PLEOMORPHIC ADENOMA OF THE PAROTID GLAND INVOLVING THE OSSEOUS EXTERNAL AUDITORY CANAL

WITH A NOTE ON THE FORAMEN OF HUSCHKE

C. ROSE RABINOV, MD

SASSAN ALAVI, MD

RINALDO F. CANALIS, MD

EDWIN J. LEE

LOS ANGELES, CALIFORNIA

A recurrence of a parotid pleomorphic adenoma presenting as an external ear canal mass is reported. The route of extension was radiographically documented as an incompletely closed foramen of Huschke. The historical and developmental features of this foramen and the mechanisms of tumor extension as they relate to it are discussed.

KEY WORDS — external auditory canal, foramen of Huschke, parotid gland, pleomorphic adenoma, recurrence.

INTRODUCTION

Curative treatment for benign pleomorphic adenomas requires resection of the tumor with a surrounding cuff of normal tissue. This approach has resulted in recurrence rates in the 3.5% range,^{1,2} significantly less than when these tumors were removed by simple enucleation. Pleomorphic adenomas are contained within a fibrous pseudocapsule, but this envelope is often incomplete and allows for tumoral projections to extend beyond its margins. Enucleation may result in shearing off these projections and seeding of the surgical field.³⁻⁵ Another mechanism for recurrence is tumor rupture and spillage of its cells.

Pleomorphic adenomas usually recur in the surgical bed. In this communication, we report on a recurrent parotid tumor presenting as an external auditory canal (EAC) mass. The pathway of tumor spread was a defect in the anterior-inferior part of the EAC, interpreted as a persistent foramen of Huschke. To our knowledge, there are no previous reports of this manner of recurrence. The history and developmental anatomy of this anomaly are discussed along with other possible routes of tumor extension.

Developmental Anatomy. The bony EAC is absent in the newborn. It develops from the lateral and upward growth of the annulus tympanicus and can be identified toward the end of the first week of life (in the term infant). It does not reach complete form until much later in childhood. The foramen of Huschke is located in the anterior-inferior surface of the tympanic plate. The middle of this surface, which forms part of the EAC, is usually thin, and it is at this level

that an opening resulting from incomplete ossification of the plate may be found.⁶

The foramen of Huschke is evident in the 12-month-old infant and usually closes by the fourth or fifth year of life.^{6,7} Persistence of the foramen of Huschke beyond childhood (Fig 1) is uncommon, but

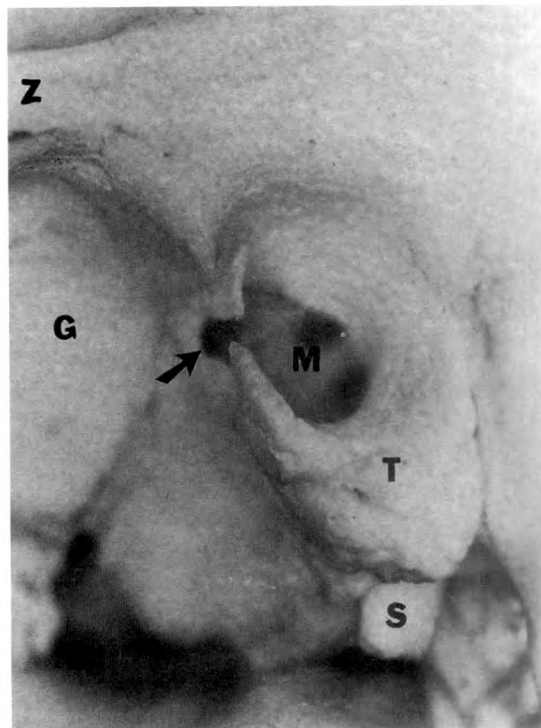


Fig 1. Photograph of lateral surface of temporal bone of adult male skull. Persistent foramen of Huschke is shown (arrow). T — tympanic bone, M — middle ear, Z — zygomatic arch, G — glenoid fossa, S — styloid process (broken).

From the Division of Head and Neck Surgery, UCLA School of Medicine, Los Angeles, and Harbor-UCLA Medical Center, Torrance, California.
CORRESPONDENCE — Rinaldo F. Canalis, MD, Division of Head and Neck Surgery, Harbor-UCLA Medical Center, 1000 W Carson St, Torrance, CA 90509.

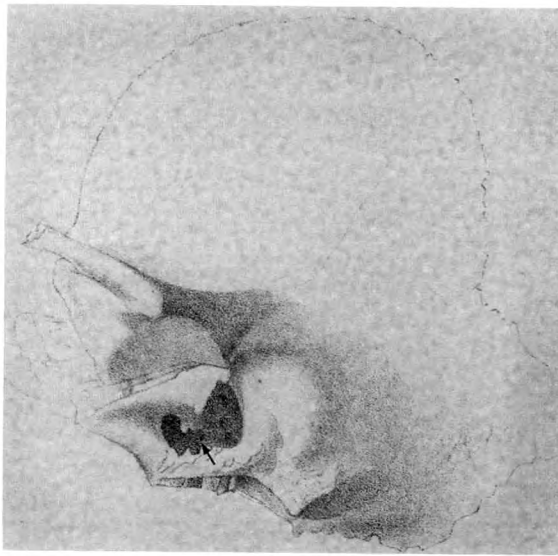


Fig 2. Von Troltsch's illustration of external ear canal in skull of 3-year-old child. Arrow (added) indicates foramen of Huschke. (Modified from von Troltsch.⁹)

its true incidence and anatomic variances have not been investigated. Documentation of the foramen of Huschke requires thin computed tomography (CT) scan sections of the tympanic bone in the sagittal projection.

Historical Note. Emil Huschke (1797-1858), originally a professor at Jena, succeeded Samuel Thomas Soemmerring at the University of Munich. Soemmerring, best known as the inventor of the electric telegraph, was responsible for an anatomic atlas of the ear (*Icones organi aditus humani*), first published in Frankfurt in 1806.⁸ Huschke revised and completed this work, which was published in Leipzig in 1844, as S. T. Soemmerring's *Textbook on the Viscera and Sensory Organs of the Human Body. Revised and Concluded by B. Huschke*. Huschke added many personal observations to the text, including descriptions of the zona dentata of the cochlea (of Huschke) and of the foramen discussed in this paper. Several interesting clinicopathologic observations are due to Huschke, including one of the first descriptions of malleal ankylosis, and two cases of stapedial fixation in deaf-mutes.

Following Huschke's description, the foramen was mentioned only occasionally by otologists of the second half of the 19th century. Von Troltsch's description is perhaps the most complete. It appeared in James Hinton's English translation in the New Sydenham Society Series of 1874.⁹ Von Troltsch did not credit Huschke's observation and remarked that "these peculiarities of the osseous meatus are very little known and as far as I know have not been specially represented."^{9(p2)} He stated that the foramen often persists until the sixth year of life and

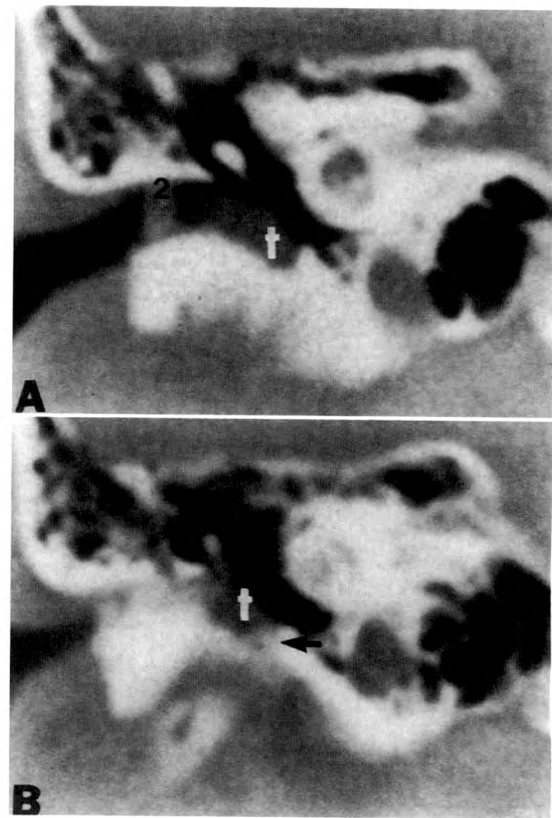


Fig 3. Coronal computed tomographic sections (2 mm apart) of right temporal bone. A) Showing two masses (2 and t) in osseous portion of external ear canal. B) Area of foramen of Huschke (arrow), and tumor (t) located in anterior-inferior portion of external ear canal.

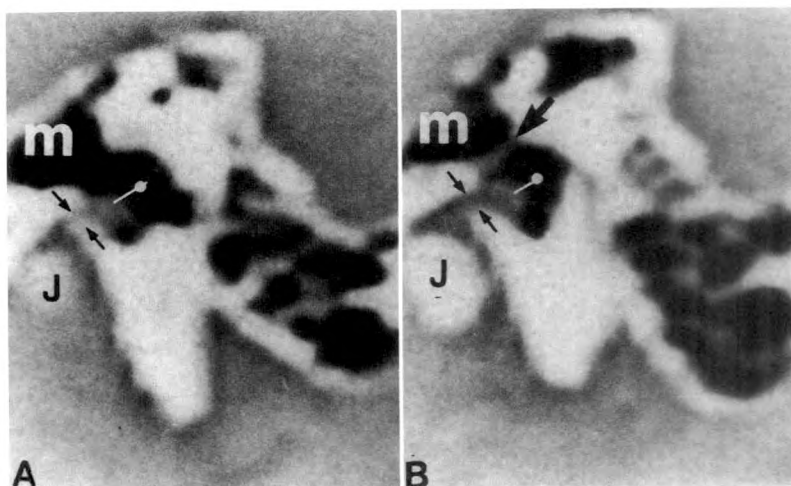
warned against interpreting it as a morbid condition. He included an illustration of the skull of a 3-year-old child, which clearly shows the foramen (Fig 2⁹).

CASE REPORT

A 54-year-old man presented to UCLA Medical Center with a 6-month history of right-sided otalgia associated with headaches and ipsilateral conductive hearing loss. His past history was significant for a right total parotidectomy for a deep lobe pleomorphic adenoma, performed 19 years previously. He had also sustained resection of a recurrence of this tumor, which involved the upper third of the masseter muscle, 12 years after the first procedure. The patient was otherwise healthy.

Examination revealed two masses in the right EAC. The first was located in the anterior wall, approximately 3 mm lateral to the pars flaccida. The second, much larger mass, was located anterior-inferiorly directly adjacent to and partially obscuring the tympanic membrane. The distance between the two masses was approximately 2 mm. Other than scars from prior surgical procedures, the remainder of the head and neck examination findings were

Fig 4. A,B) Sagittal plane reformats (2 mm apart) of right temporal bone computed tomography scan. Small arrows show limits of foramen of Huschke. Note tumor extending through it. Pin shows tumor in external ear canal. Large arrow — tympanic membrane, m — middle ear, J — condyle of mandible.



unremarkable. No masses were palpable in the parotid region, and facial nerve function was intact.

A preoperative CT scan of the temporal bone showed a homogeneous soft tissue density in the medial aspect of the right EAC, abutting the tympanic membrane, and a smaller, sessile mass in close proximity to it. Coronal sections showed an area of possible dehiscence in the anterior-inferior portion of the EAC (Fig 3). Reformats of these sections in the sagittal plane demonstrated an anterior-inferior bone defect. The limits of this opening appeared smooth. The tumor was noted to extend through this opening, which was diagnosed as an incompletely closed foramen of Huschke (Fig 4). The left temporal bone was entirely normal. Gadolinium contrast magnetic resonance imaging demonstrated both masses (Fig 5). No lesions were noted in the parotid area.

Surgical exploration was recommended. The smaller mass was taken for biopsy by amputation at its base. The medial mass was found to be firm and tightly adherent to the anterior-inferior canal wall. A plane could be developed between the mass and the tympanic membrane, but an attempt at separation of the tumor from the adjacent canal wall provoked an im-



Fig 5. Gadolinium contrast magnetic resonance imaging demonstrating close proximity of external ear canal masses (t).

mediate response from the facial nerve monitor. Low-voltage (1 mA) stimulation of the mass consistently produced facial activity, suggesting that the tumor was either in direct contact with or in very close proximity to the facial nerve. It was decided that complete excision would require exposure and preservation of the peripheral facial nerve and lateral temporal bone resection. The procedure was terminated pending further discussion with the patient. Frozen and permanent histopathologic sections

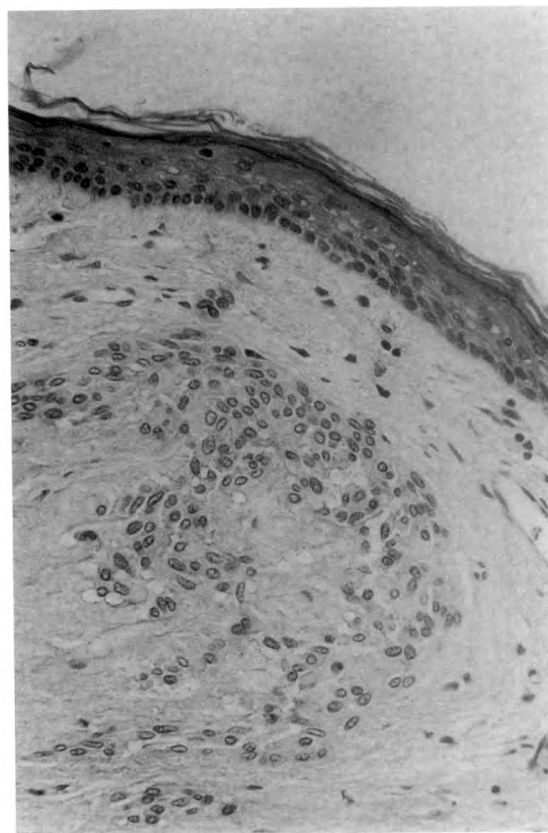


Fig 6. Microphotograph of superior external ear canal mass biopsy demonstrating benign pleomorphic adenoma (H & E, original x56). Note that tumor is covered by normal skin.

showed a subcutaneous nodule and confirmed the diagnosis of pleomorphic adenoma (Fig 6).

The patient refused consent for lateral temporal bone resection because of religious beliefs regarding the possible need for a blood transfusion. Eventually, he was operated on elsewhere, where he underwent excision of the portion of tumor involving the ear canal. Resection encompassed the skin from which the smaller lesion had been amputated, but not the parotid bed. Subcutaneous microscopic extension into the ear canal beyond obvious gross tumor was noted. No lesions were found at middle ear exploration. Facial nerve function was preserved.

DISCUSSION

The clinical and radiographic findings of the case presented are essentially diagnostic of a recurrent parotid pleomorphic adenoma. However, the unusual presentation of this tumor mandates clarification of several issues, to be discussed below. Did the primary tumor become more aggressive or malignant? Was the tumor a primary lesion of the EAC? What routes, other than the foramen of Huschke, could have favored this manner of tumor extension?

The tumor initially arose in the deep lobe of the right parotid gland. It is unknown whether there was any spillage or violation of the pseudocapsule at the first operation. Histopathologic findings on the primary tumor and recurrences are all consistent with a benign, noninfiltrative tumor. The timing of recurrences, 12 and 19 years after primary resection, further supports a nonmalignant lesion.

Primary adenomas of the EAC may be indistinguishable from those arising from the salivary glands. The skin lining the osseous part of the canal lacks hair follicles, as well as sebaceous and ceruminous glands, whereas that covering the cartilaginous part contains these elements.^{10,11} This variance accounts for the prevalence of primary glandular tumors in the lateral, rather than the medial, portion of the EAC.¹² In our case, the tumor location within the osseous part of the canal makes the possibility of a primary adenoma unlikely.

The history of ipsilateral primary and recurrent parotid pleomorphic adenomas strongly suggests

further extension of the disease to the EAC. Potential routes of spread from the primary site include the fallopian canal, cartilaginous fissures, the petrotympanic suture, and tracts resulting from blunt or iatrogenic trauma. Only the last three merit consideration in our case.

Piercing through the lateral cartilaginous canal are two or three vertical openings known as the fissures of Santorini. These natural defects are filled with connective tissue and provide passage for small blood vessels.⁷ Infection progressing through the fissures of Santorini is frequently seen in necrotizing otitis externa,¹³ but the lateral position of the fissures excludes them as vehicles for tumor extension in this case. To our knowledge, dehiscence of the petrotympanic suture has not been reported. Tumor progression through this suture would likely result in middle ear involvement, which was absent in this case.

The possibility that tumor spread through a false passage is an appealing theory. Dissection of the cartilaginous canal and exposure of the "pointer" is a routine step in parotidectomy. Separation of the osteocartilaginous junction, and skin dehiscence can occur if canal retraction is extreme. Such a false passage associated with tumor spillage could result in tumor entrapment and eventual extension to the temporal bone. However, the location of the recurrence deep to the osteocartilaginous junction, and the integrity of the canal skin at this level exclude this possibility.

CONCLUSION

Analysis of the case presented leaves an incompletely obliterated foramen of Huschke as the most likely pathway for tumor extension. The CT sections in the sagittal plane clearly show a defect in the anterior-inferior part of the tympanic bone, below the petrotympanic suture. The edges of this defect are smooth and straight, suggesting a natural opening rather than tumoral erosion. The past history of primary and recurrent parotid tumors and the location of the masses in the osseous portion of the EAC make the existence of a primary lesion of this site, or neoplastic implantation through an iatrogenic passage, an unlikely possibility.

ACKNOWLEDGMENT — Dr Robert Lufkin evaluated the radiographic findings.

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TWENTIETH ANNUAL MEETING OF THE BÁRÁNY SOCIETY

The Twentieth Annual Meeting of the Bárány Society will be held in Würzburg, Germany, September 20-23, 1998. For more information, contact the Local Organizing Committee, Institute of the 4-G-F, Kurhausstrasse 12, D 97688 Bad Kissingen, Germany; telephone 0049/971-64832; fax 0049/ 971-68637.

CROSS-INNervation OF THE THYROARYTENOID MUSCLE BY A BRANCH FROM THE EXTERNAL DIVISION OF THE SUPERIOR LARYNGEAL NERVE

SINA NASRI, MD

LAS VEGAS, NEVADA

JOEL A. SERCARZ, MD

LOS ANGELES, CALIFORNIA

POUNEH BEIZAI

LOS ANGELES, CALIFORNIA

YOUNG-MO KIM, MD

LOS ANGELES, CALIFORNIA

MING YE, MD

LOS ANGELES, CALIFORNIA

GERALD S. BERKE, MD

LOS ANGELES, CALIFORNIA

The neuroanatomy of the larynx was explored in seven dogs to assess whether there is motor innervation to the thyroarytenoid (TA) muscle from the external division of the superior laryngeal nerve (ExSLN). In 3 animals, such innervation was identified. Electrical stimulation of microelectrodes applied to the ExSLN resulted in contraction of the TA muscle, indicating that this nerve is motor in function. This was confirmed by electromyographic recordings from the TA muscle. Videolaryngostroboscopy revealed improvement in vocal fold vibration following stimulation of the ExSLN compared to without it. Previously, the TA muscle was thought to be innervated solely by the recurrent laryngeal nerve. This additional pathway from the ExSLN to the TA muscle may have important clinical implications in the treatment of neurologic laryngeal disorders such as adductor spasmodic dysphonia.

KEY WORDS — adductor spasmodic dysphonia, in vivo canine laryngeal model, laryngeal innervation, recurrent laryngeal nerve, superior laryngeal nerve, thyroarytenoid muscle.

INTRODUCTION

The innervation pattern of the intrinsic laryngeal muscles was initially determined decades ago, with anatomic, histologic, and degenerative studies.^{1,2} With the exception of the cricothyroid (CT) muscle, all intrinsic laryngeal muscles are innervated by branches of the recurrent laryngeal nerve (RLN). The CT muscle is innervated by the external branch of the superior laryngeal nerve (ExSLN). Due to limitations of past techniques, the exact branching patterns of small nerves could not be identified. During dissection, it is difficult to distinguish small nerves from blood vessels and connective tissue. Moreover, distal branches are prone to injury during anatomic studies of nerves. Today, more sophisticated histologic methods and microdissection techniques are available to assess the branching patterns of the laryngeal nerves.

The anatomy and physiology of the ExSLN have been controversial. In 1932, Lemere¹ reexamined the innervation pattern of the canine larynx. Anatomically, he discovered that the ExSLN supplied the CT muscle and then pierced the thyroarytenoid (TA) muscle to innervate the mucosa beneath the vocal

folds anteriorly. He believed that the ExSLN fibers innervated the TA muscle as they passed through it. Electrical stimulation of the ExSLN did not, however, produce activity in the TA muscle on electromyography (EMG). Lemere was unsuccessful in finding *physiological* evidence of TA innervation by the ExSLN fibers.

In 1993, Sanders et al³ demonstrated anatomically that the ExSLN may innervate the TA muscle. With Sihler's staining technique, a cross-innervation between RLN terminals and the ExSLN in the TA muscles was found in 4 of 10 excised canine larynges. The ExSLN passed from the posterior surface of the CT muscle across the pyriform sinus and entered the lateral surface of the TA muscle. Since Sihler's method can only provide an anatomic proof of innervation patterns, Sanders et al were unable to determine whether the connection between the ExSLN and the TA muscle contained motor fibers.

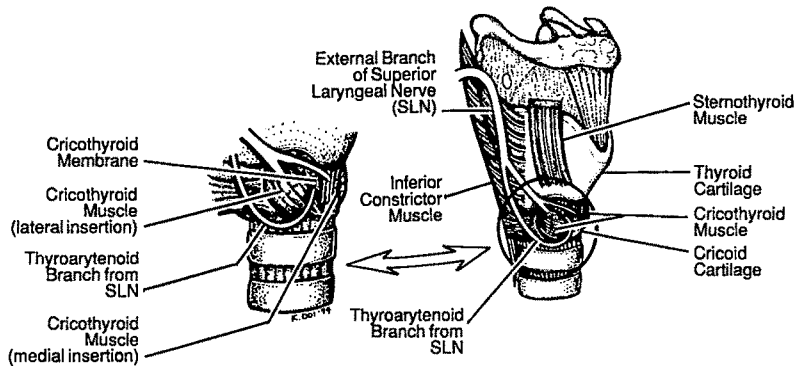
The present experiment examined the function and branching pattern of the distal ExSLN in an in vivo canine model. Microanatomic dissection of 7 canine larynges was performed. Electrodes were applied to

From the Division of Head and Neck Surgery, Stanford Medical Center, Stanford (Nasri), the Division of Head and Neck Surgery, University of California-Los Angeles School of Medicine, Los Angeles (Beizai, Ye, Sercarz, Kim, Berke), and the Division of Audiology and Speech Pathology, Wadsworth Veterans Administration Medical Center, West Los Angeles (Ye), California. This research was supported by National Institutes of Health/National Institute on Deafness and Other Communication Disorders grant R01 DC0085-01 and by Veterans Administration Merit Review Funds. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California-Los Angeles.

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CORRESPONDENCE — Sina Nasri, MD, 3101 S Maryland Pkwy, Ste 102, Las Vegas, NV 89109.

Fig 1. Schematic of thyroarytenoid (TA) branch takeoff from external division of superior laryngeal nerve (ExSLN). Enlarged segment on left side of Figure demonstrates point of entry of TA branch of ExSLN between medial and lateral insertions of cricothyroid muscle. SLN—superior laryngeal nerve.



the dissected nerves in an attempt to produce muscular contraction. The function was confirmed with EMG recordings from the muscles and by videostroboscopic evaluation of the larynges.

MATERIALS AND METHODS

In Vivo Canine Model of Phonation. The in vivo canine model of phonation has been used in our laboratories extensively, and a detailed description has been published.⁴ Each animal was initially premedicated with 3 mL of acepromazine maleate intramuscularly. Intravenous pentobarbital sodium (Nembutal) was administered to result in corneal anesthesia. Additional Nembutal was given to maintain this level of anesthesia.

The animal was placed supine on the operating table, and a midline incision was made to expose the trachea from the sternal notch to the hyoid bone. The strap and sternocleidomastoid muscles were retracted laterally to expose the larynx and trachea. A low tracheostomy was performed at the level of the suprasternal notch, through which an endotracheal tube was passed to allow ventilator-assisted respiration. A second tracheostomy was performed in a more cephalad position, through which an endotracheal tube was placed.

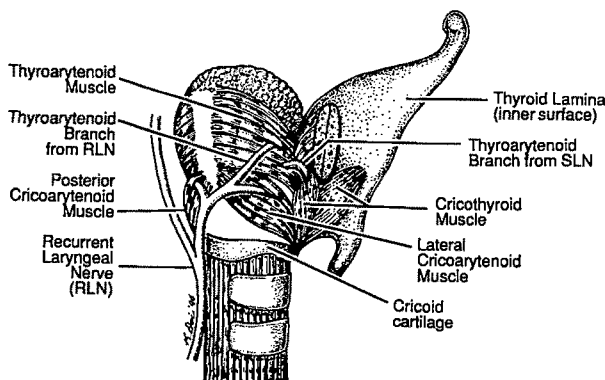


Fig 2. View of intrinsic laryngeal muscles demonstrating TA branches from SLN and recurrent laryngeal nerve (RLN). Thyroid lamina is outfractured to examine muscles present on its inner surface.

The tip of the upper endotracheal tube was positioned approximately 10 cm below the level of the glottis. The cuff was inflated to just seal the trachea. The air was heated and humidified by passing through 5 cm of heated water. It was then passed through the upper endotracheal tube to establish a constant flow system. The temperature of air was 37°C when measured at the glottic outlet. The amount of flow was controlled with a Gilmont flowmeter. Direct visualization of the larynx was possible when the epiglottis was suspended from a fixed point with a 1-cm button.

Bilateral RLNs and ExSLNs were isolated. The RLNs were exposed 5 cm inferior to the larynx in the tracheoesophageal groove; their identity was confirmed with electrical stimulation. A thyroid cartilage window was made to aid in identification of any nerve branches entering or leaving the TA muscle. Under microscopic examination, the TA branch of the RLN was identified as the last terminal branch and its identity was confirmed by electrical stimulation; it was cut distally from the branching point, with care to avoid injury to the other terminal branches.

The ExSLN was isolated at the level of the superior attachment of the sternothyroid muscle to the thyroid cartilage (Fig 1). The edge of the thyroid cartilage was lifted and the pharyngeal constrictor muscle was transected. Next, the CT joint was dislocated and any nerve traveling between the medial and lateral insertions of the CT muscle entering the TA muscle was microdissected (Figs 1 and 2). The nerve was then followed retrograde to verify its origin from the ExSLN.

One electrode was applied to each trunk of the RLN, approximately 5 cm proximal to the takeoff of the posterior division (hereinafter referred to as the "trunk"). The second electrode was applied to the ExSLN nerve branch to the TA muscle. The electrodes were isolated such that they were contacting only the nerve branches and not the muscles. Electrical input to the superior laryngeal nerve (SLN) electrode was supplied by a Grass model 54H stimulator (Grass Instruments, Quincy, Mass). Current stimula-

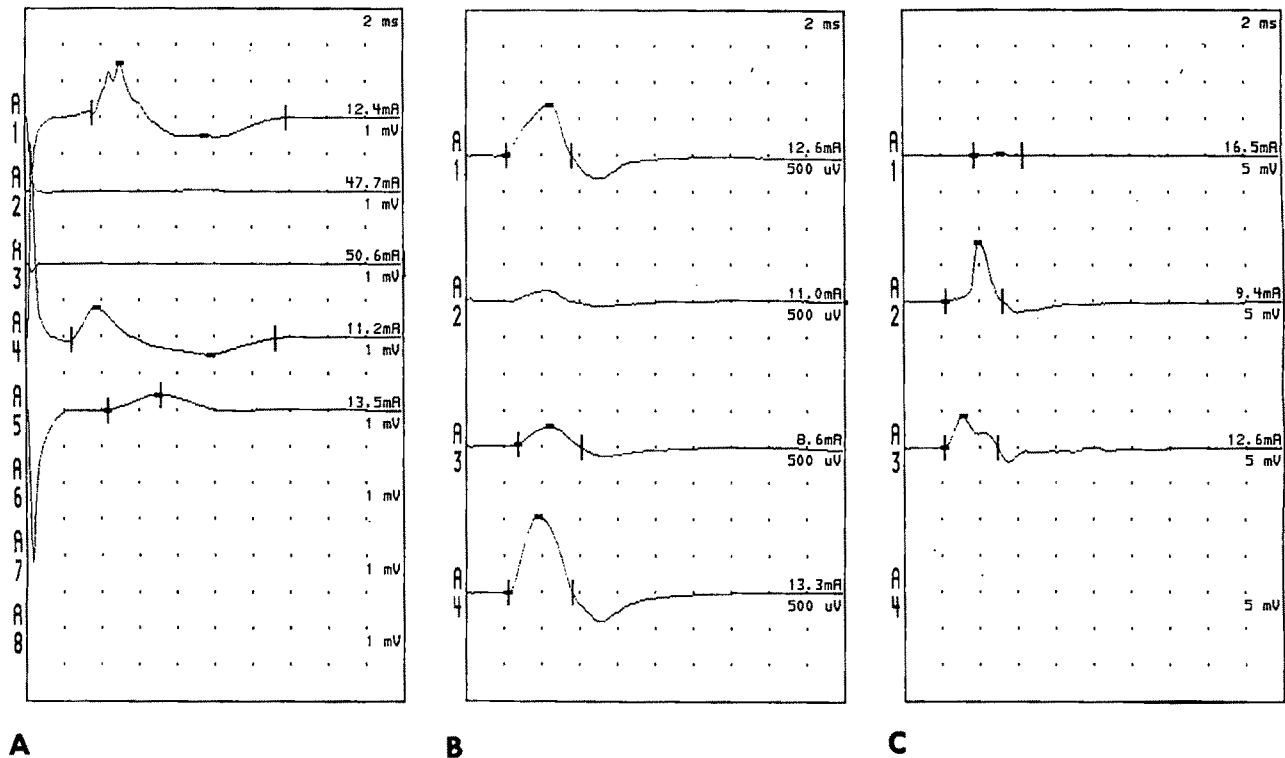


Fig 3. Evoked electromyography tracings from TA muscle. X-axis is time (2 milliseconds per division), and y-axis is amplitude. Intensity of stimulating current is shown in milliamperes. A) Stimulation of TA branch of RLN (A1), RLN "trunk" (A2), internal division of SLN (A3), TA branch of ExSLN (A4), and ExSLN trunk (A5). For amplitude (y-axis), there is 1 mV per division. B) Stimulation of ipsilateral ExSLN trunk (A1) and ipsilateral TA branch of ExSLN (A2 through A4). Recordings were obtained from posterior TA muscle (A2), middle TA muscle (A3), and anterior TA muscle (A4). For amplitude (y-axis), there is 0.5 mV per division. C) Stimulation of contralateral TA branch of ExSLN. Recordings were obtained from posterior TA muscle (A1), anterior TA muscle (A2), and middle TA muscle (A3). For amplitude (y-axis), there are 5 mV per division.

tion to the RLN trunk was provided by a constant current stimulator (WR Medical Electronics RLN Stimulator, model S2LH, St Paul, Minn). The frequency of nerve stimulation was 80 Hz with a pulse duration of 1.5 milliseconds. The intensities used for stimulation ranged from 1.5 to 3.0 V. In order to avoid problems associated with ground loops, the measuring instruments were floated.

Evoked Electromyographic Studies. Evoked EMG was recorded during experimental trials from the TA muscle during stimulation of the ipsilateral and contralateral TA branches of the RLN, as well as the ExSLN trunk and the branch to the TA muscle. The placement was confirmed by stimulating the TA branch of the RLN and recording the EMG signal from the muscle. Stimulation of other branches of the RLN did not result in a recorded EMG signal from the TA muscle. Bipolar concentric EMG needle electrodes were placed transcervically into the TA muscle at the anterior, middle, and posterior thirds of the muscle. The signal was recorded with a Nicolet Viking II EMG instrument. The signal was high-pass-filtered at 20 Hz and low-pass-filtered at 10 kHz. The output was digitized at 20 kHz and stored.

Videolaryngostroboscopy. After the TA branch of

the RLN was transected, stroboscopic images of vocal fold movement were obtained during stimulation of the RLN trunk with and without stimulation of the ExSLN. These images were recorded with a Storz laryngostroboscope (model 8000). A Storz 0° telescope was connected to the stroboscope via fluid-filled cables. The image from the telescope was recorded with a Storz CCD (charge-coupled device) video camera (model 9000) and a Sony U-matic videocassette recorder (VO-5800).

Experimental Design. The anatomic microdissection was performed as previously described. Stimulating electrodes were applied to the RLN "trunk" (following transection of the TA branch) and to the main ExSLN, as well as its branch(es) to the TA muscle. First, the TA branches of the ExSLN were stimulated, and any activity of the TA muscle was videorecorded. In addition, EMG recordings were obtained from the muscle. Next, all bellies of the CT muscle were transected, and the videolaryngoscopic and EMG recordings were obtained from the TA muscle during stimulation of the main trunk of the ExSLN. To ensure that this response was not antidromic, the nerve was transected proximal to the site of application of the microelectrode. Finally, the

RLN trunk electrode was stimulated and TA activity was recorded. Videostroboscopy was performed with stimulation of the RLN trunk with or without stimulation of the TA branch of the ExSLN. To avoid volume-conducted potentials, all ExSLN fibers not innervating the TA muscle were transected.

RESULTS

Anatomic Study. Three of 7 animals studied revealed TA branches from the ExSLN. In 2 of the animals, this innervation pattern was bilateral. One of 2 dogs with bilateral TA innervation from the ExSLN had two branches on each side. The other dog had one branch on one side and two branches on the other side. The animal with unilateral innervation had two branches from the ExSLN to the TA muscle.

Physiological Study. Bilateral stimulation of the RLN trunks did not result in phonation. However, in 2 animals with bilateral TA branches from the ExSLN, after simultaneous stimulation of bilateral RLN trunks and TA branches of the ExSLN, phonation ensued, including the presence of mucosal traveling waves. The bulk of the activity of the TA branch of the ExSLN was observed in the anterior third of the vocal fold, although there was some activity posteriorly when the ipsilateral ExSLN TA branch was stimulated. Stimulation of the contralateral TA branch of the ExSLN resulted in slight bulging of the TA muscle only at the anterior third of the muscle.

Electromyographic Study. Figure 3A reveals the EMG recordings from the TA muscle during stimulation of the TA branch from the RLN (A1), the RLN trunk (A2), the internal division of the SLN (A3), the branch from the ExSLN directly to the TA muscle (A4), and the trunk of the ExSLN (A5). The peak-to-peak amplitudes are 2,058, 0, 0, 1,305, and 437.8 μV , respectively. To ensure absence of field artifact and antidromic response, electrodes were isolated from the surrounding tissues. In addition, the experiment was repeated with the contralateral ExSLN transected as well as intact. Finally, the bellies of the CT muscle were transected and the ExSLN was cut proximal to the site of placement of the stimulating electrode.

In Fig 3B, the ExSLN trunk (A1) or the ExSLN branch to the TA muscle (A2 through A4) was stimulated and EMG recordings were obtained from the anterior ipsilateral TA muscle (A1 and A4), the posterior ipsilateral TA muscle (A2), and the mid-ipsilateral TA muscle (A3). The peak-to-peak amplitudes of the EMG signal in A1 through A4 were 701, 111, 245, and 1,057 μV , respectively. The areas under the graphs for the signals were 1,431, 245, 613,

and 2,104 μV times milliseconds. It is important to note that simultaneous EMG recordings from other intrinsic laryngeal muscles revealed no electrical activity.

Figure 3C shows the ExSLN branch to the TA muscle being stimulated and the EMG recordings obtained from the anterior (A2), middle (A3), and posterior (A1) parts of the contralateral TA muscle. The EMG recording is strongest anteriorly and becomes weaker as one moves posteriorly.

DISCUSSION

This in vivo study was designed to test the hypothesis, based on previous observations, that motor innervation from the ExSLN to the TA muscle is present in the dog.³ It has been accepted until recently that the TA, posterior cricoarytenoid, interarytenoid, and lateral cricoarytenoid muscles are innervated solely by the RLN, and that the CT muscle is supplied by the external division of the SLN exclusively.^{5,6}

There has been some controversy in the past regarding the pattern of laryngeal innervation. Using dissection and degenerative studies, Exner⁷ showed that every laryngeal muscle was innervated bilaterally, by both the SLN and the RLN. New⁸ discovered that the internal division of the SLN provides motor innervation to the interarytenoid muscle. Previous studies have relied on anatomic evidence to determine the innervation of the TA muscle.^{1,3} Therefore, there is no proof of the motor or sensory nature of this innervation. In this study, anatomic and physiological techniques were used to prove that similar to the TA branch of the RLN, the ExSLN supplies motor innervation to the TA muscle.

In 2 of 7 dogs examined, bilateral innervation from the ExSLN to the TA muscle was present. In 1 animal, this innervation was unilateral. Four dogs did not demonstrate a branch from the ExSLN. Whether this variability represents actual anatomic differences or the difficulty in microdissection of these small nerve branches awaits future investigation. However, the variability found in this study is in agreement with the findings of a previous histologic work.³

The EMG signals were recorded in the TA muscle after stimulation of the terminal ExSLN branches to the TA muscle. Evoked EMG is widely used as a tool for determining the function of a muscle in response to stimulation of the motor neurons supplying it.^{9,10} The shape and size of the EMG signal can be used to determine the motor nature of the neuronal input.⁹ The signals recorded in this study from the TA muscle during stimulation of the ExSLN branches

identify these branches as motor.

The activity of the TA muscle is essential for normal vocal fold vibration and voice production. When activated, this muscle causes a change in vocal fold stiffness and hence the fundamental frequency.^{11,12} After the TA branch of the RLN was cut, the RLN "trunk" was stimulated. The resultant phonation was hoarse and the mucosal traveling waves were absent because of the lack of complete closure of the folds. When the RLN trunk was stimulated simultaneously with the bilateral ExSLN branches to the TA muscle, phonation significantly improved. Videolaryngostroboscopy revealed presence of a mucosal traveling wave after stimulation of the bilateral ExSLN branches.

It appears that the bulk of activity of the TA branch of the ExSLN is located in the anterior third of the vocal fold. The EMG recordings showed that as one moved posteriorly along the TA muscle, the peak-to-peak amplitude of the measured signal decreased in size. This may be important in intricate control of the pitch, an important function of the TA muscle.

The fact that the TA has additional motor innervation has many important implications in treating patients with certain voice disorders, such as spasmodic dysphonia. This speech disorder is a localized

dystonia, producing hyperadduction of the vocal folds during speech and a halting, strained vocal pattern. The additional innervation may explain why some patients have not had long-term control of adductor spasmodic dysphonia following TA denervation.¹³ An attempt will be made in the design of upcoming surgical procedures to identify and section the TA branch from the ExSLN as well as that from the RLN. These patients will then be followed to observe the long-term effect of surgery on control of spasmodic dysphonia.

It should be emphasized that there are some differences between canine and human laryngeal innervation. As an example, in some dogs, there may be another major branch of the RLN, the pararecurrent nerve, that is not observed in humans.¹ Furthermore, both in dogs and in humans, individual variations in the anatomy of the RLN and SLN are fairly common.

In addition to design of new surgical procedures, future studies would include retrograde staining of the TA nerve branches from the RLN and SLN in order to determine their central projection from the nucleus ambiguus. These centers can then be compared to one another and be used to develop a detailed map of the laryngeal motor centers within the nucleus ambiguus. These histologic studies are currently under way in our laboratories.

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OTOTOXIC EFFECT OF ERYTHROMYCIN ON COCHLEAR POTENTIALS IN THE GUINEA PIG

TOSHIMITSU KOBAYASHI, MD
NAGASAKI, JAPAN

YOU RONG, MD
TANJIN, PEOPLE'S REPUBLIC OF CHINA

TOSHIHIKO CHIBA, MD
SENDAI, JAPAN

DANIEL C. MARCUS, DSC
OMAHA, NEBRASKA

KENJI OHYAMA, MD
SENDAI, JAPAN

TOMONORI TAKASAKA, MD
SENDAI, JAPAN

The mechanism of hearing loss due to the administration of intravenous erythromycin was investigated in the albino guinea pig, and it was found for the first time that this drug causes cochlear dysfunction. The endocochlear potential (EP) and the cochlear microphonics (CM) recorded at the first cochlear turn transiently decreased when erythromycin was administered intravenously at dosages of 100 and 150 mg/kg. The averaged maximum decrease in EP was 16 mV ($n = 5$) and 33 mV ($n = 5$) for 100 and 150 mg/kg, respectively. The maximum decrease in the CM was about 25% when the EP reached its lowest value with the injection of 150 mg/kg. A complete recovery of the EP and CM ensued within 20 minutes after each erythromycin dose. The perilymphatic perfusion of 3 mmol/L of erythromycin decreased the EP and CM; however, in contrast to the intravenous administration, the decrease of the CM was nearly complete and both the EP and CM were irreversible. Hearing loss due to intravenously administered erythromycin could likely be attributable to the transient dysfunction of the stria vascularis, although concomitant dysfunction of the central auditory pathway cannot be excluded.

KEY WORDS — cochlear microphonics, endocochlear potential, erythromycin, hearing loss, ototoxicity.

INTRODUCTION

Erythromycin is a macrolide antibiotic, discovered by McGuire et al¹ in 1952, which is effective against infections attributable to *Mycoplasma pneumoniae* or *Legionella pneumophila* and is also widely used for penicillin-allergic patients infected by *Streptococcus*. It has been widely used for community-acquired pneumonia, and in recent years, its use for the treatment of panbronchiolitis, chronic sinusitis, and secretory otitis media has also been tried with low-dose protocols.^{2,3} Although one of its advantages is that serious adverse reactions are rare, there is growing clinical evidence that this drug can cause hearing loss.⁴

The first report of erythromycin ototoxicity was made by Mintz et al⁵ in 1973, with regard to two females who had been treated for pneumonia with erythromycin lactobionate intravenously. Bilateral perceptive deafness, on average 50 to 55 dB, returned to normal decibel averages of 15 dB after the drug was changed from erythromycin to cephalothin. Since this report, about 50 cases of bilateral sensorineural hearing loss, typically uniform across frequencies, have been reported in association with high-dose intravenous or even oral administration of erythro-

mycin.⁴

Stupp et al⁶ found considerable hair cell loss after intratympanic administration of erythromycin, but the doses were very high and the route was different from that in clinical practice.

The mechanism by which erythromycin causes ototoxicity has not been fully elucidated. Brummett⁴ reported that anesthetized guinea pigs receiving erythromycin lactobionate (125 mg/kg per hour) showed a change in the auditory brain stem response (ABR) to 8-kHz tone pulses. The first effect to be found was an increase in latency of the fourth wave, followed in sequence by the other waves. Brummett⁴ also reported that human patients receiving erythromycin experienced an increased latency in wave V of the ABR with no changes in the earlier waves. These data were interpreted by the author to show that erythromycin ototoxicity may be due to an effect on the central auditory pathway. This hypothesis is strengthened by several other reports of adverse effects of erythromycin on the central nervous system.⁴

In contrast, Sacristan et al⁷ reported an absence of waves I to III of the ABR during treatment with erythromycin and proposed dysfunction at the more

From the Department of Otolaryngology, Tohoku University School of Medicine (Kobayashi, Rong, Chiba, Takasaka), and the Department of Otolaryngology, Tohoku Rosai Hospital (Ohayama), Sendai, Japan, and the Biophysics Laboratory, Boys Town National Research Hospital, Omaha, Nebraska (Marcus). This work was supported by a Scientific Research Grant from the Japanese Ministry of Education (B-06454482).

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CORRESPONDENCE — Toshimitsu Kobayashi, MD, Dept of Otolaryngology, Nagasaki University School of Medicine, 1-7-1, Sakamoto, Nagasaki 852, Japan.

COMPOSITION OF ARTIFICIAL PERILYMPH
PRODUCED FOR EXPERIMENT

	mmol/L
Sodium chloride	125.0
Potassium chloride	3.5
Sodium bicarbonate	25.0
Calcium chloride	1.3
Magnesium chloride (hexahydrate)	1.1
Monosodium phosphate (monobasic, monohydrate)	0.5
Glucose	3.3
Urea	2.1

peripheral portion of the auditory pathway.

As the site responsible for hearing loss due to erythromycin is thus controversial, the present study was designed to elucidate whether or not the cochlea is a target of erythromycin ototoxicity.

MATERIALS AND METHODS

Albino guinea pigs with body weights of 200 to 400 g were used. The animals were anesthetized with 30 mg/kg of pentobarbital sodium (Nembutal), immobilized by suxamethonium chloride (Relaxin), and kept under artificial respiration. The cochlea was exposed by a ventral approach. Recordings of the cochlear potentials (endocochlear potential [EP] and cochlear microphonics [CM]) were made by a previously reported method used in earlier studies of cochlear pathophysiology.⁸⁻¹⁰ A glass microelectrode (tip 2 to 3 μ m) filled with 150 mmol/L potassium chloride was inserted into the scala media of the first or the second cochlear turn through either the round window membrane or a small hole created in the lateral cochlear wall; the microelectrode was connected to a WPI FD223 amplifier. The CM was elicited by a 1-kHz, 60 dB sound pressure level (SPL)

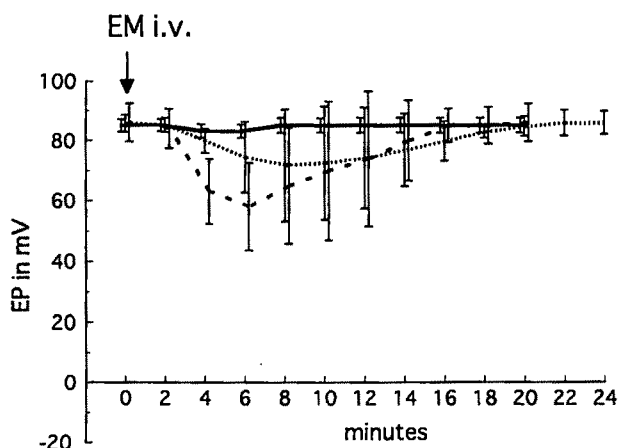


Fig 1. Averaged change of endocochlear potential (EP) recorded at first turn of cochlea in response to intravenously (i.v.) applied erythromycin (EM). Each vertical bar represents average $\pm$ 1 SD. Solid line — 50 mg/kg ($n=3$); thin dotted line — 100 mg/kg ($n=5$); thick dotted line — 150 mg/kg ($n=5$).

pure tone generated with an insert earphone (Ety-motic, ER-3A) and was measured with a lock-in amplifier (NF5610B). Erythromycin lactobionate was dissolved in distilled water at a concentration of 50 mg/mL. When used for intravenous injection, the solution was diluted with an equal amount of saline. When used as perilymphatic perfusate, the solution was dissolved in salt solution to obtain the composition of perilymph (see Table).

Intravenous injection of the erythromycin was performed slowly through a polyethylene catheter inserted in one of the jugular veins of the animal at doses of 50 and 100 mg/kg at a rate of about 50 mg/min. In experiments in which larger doses of erythromycin were used, the injection was performed via the femoral vein to reduce the direct action of the drug on the cardiopulmonary function of the animal.

Perilymphatic perfusion of the erythromycin was performed with a syringe pump (Narishige, Tokyo, Japan) at a flow rate of 10 μ L/min; a hole created in the scala tympani of the first turn of the cochlea acted as the inlet, and one made in the scala vestibuli of the first turn served as the outlet. This flow rate has been confirmed to be a relatively safe perfusion speed for avoiding damage to cochlear function.¹⁰ Prior to loading the perfusion system, the perfusate was equilibrated with a gas mixture of 95% oxygen–5% carbon dioxide. In most experiments, anoxia was induced at the end of the experiment by stopping the respirator to examine the response pattern of the EP to anoxia. During the experiments, the carbon dioxide concentration of the exhaled gas of the animal was monitored to maintain the animal's general condition.

RESULTS

Intravenously administered erythromycin reduced the EP (Figs 1 and 2). The degree of decrease in EP

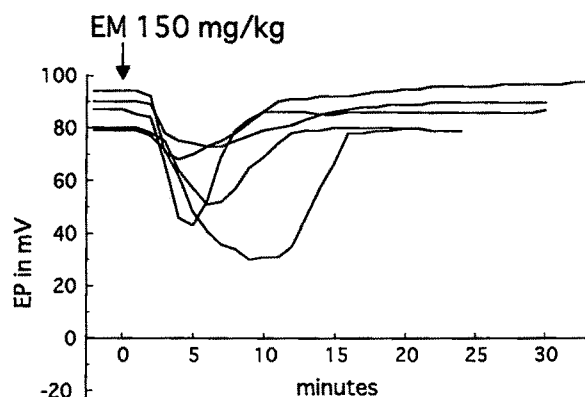


Fig 2. Change of EP recorded at first turn of cochlea in response to 150 mg/kg of intravenously administered EM ($n=5$).

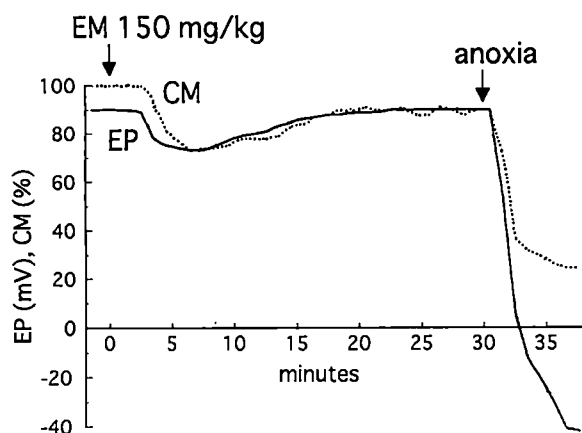


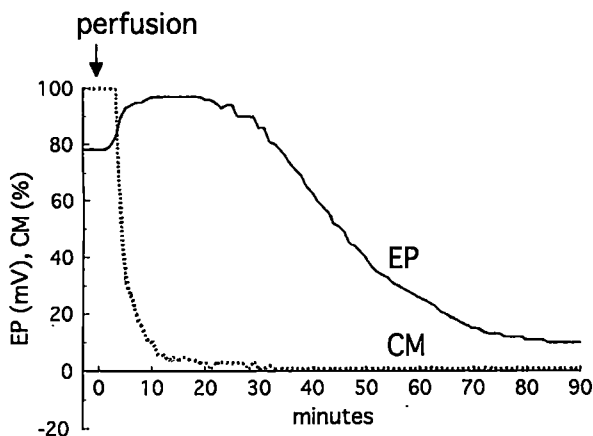
Fig 3. Representative example of simultaneous EP and cochlear microphonics (CM) recorded at first turn of cochlea in response to 150 mg/kg of intravenously administered EM.

correlated with the dose of erythromycin administered. The decrease of the EP commenced at 2.5 minutes after the start of intravenous injection of erythromycin, with the lowest values being obtained at about 5 to 10 minutes after the injection. Although 50 mg/kg of erythromycin had little effect on the EP, higher doses of 100 and 150 mg/kg of the drug caused the EP to decrease, and the averaged maximum decreases at these levels were 15.6 ± 19.4 mV ($n = 5$) and 33.0 ± 13.2 mV ($n = 5$), respectively.

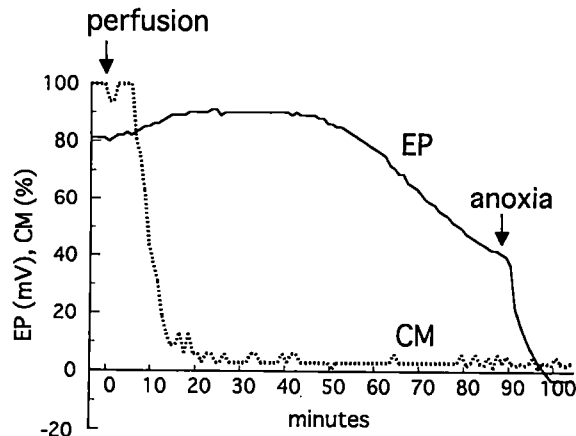
Recovery of the EP ensued after a few minutes at its lowest value, and the EP returned to its original value within 20 minutes after the injection. Figure 3 is a representative recording in which EP and CM were simultaneously recorded during intravenous application of erythromycin at a dose of 150 mg/kg ($n = 3$). The decrease in the CM was observed to be accompanied by a corresponding decrease in the EP. When the EP reached its lowest value, the CM was

reduced by about 25%. Anoxia was induced by stopping the respirator at the end of the experiment, when both the EP and CM were in full recovery from the effects of erythromycin. The EP showed a value of -42 mV at about 7 minutes after the induction of anoxia. In another experiment with 100 mg/kg of erythromycin, the negative EP obtained after similar treatment was -46 mV. These values of negative EP after anoxia were comparable to those of normal animals previously studied ($n = 7$), -38.7 ± 3.7 mV.¹¹

A decrease of the cochlear potentials was also observed in the case of perilymphatic perfusion with 3 mmol/L ($n = 4$) of erythromycin. Representative recordings of the effect of perilymphatically applied erythromycin on the EP and CM are shown in Fig 4. Both the EP and CM recorded from the second turn decreased as a result of perfusion with artificial perilymph containing 3 mmol/L of erythromycin, although the pattern of decline was different from that of intravenous administration. The EP initially increased by 10 to 20 mV and stabilized at these values for about 10 to 25 minutes before gradually declining toward 0 mV at a rate of about 1 to 2 mV/min. The change in the CM was found to be more drastic than that of the EP; the CM started to decline shortly after the start of perfusion and rapidly deteriorated to a noise level within 20 minutes after the start of perfusion. When anoxia was induced before full suppression of the EP by erythromycin (Fig 4), although the decline of the EP was accelerated, the EP did not reach full negative value, in contrast to results of the same treatment performed after intravenous administration of the drug. Similar abolishment or suppression by erythromycin of the negative EP in response to anoxia was consistently seen in other experiments as well.



A



B

Fig 4. Two representative recordings of EP and CM in response to perilymphatic perfusion with 3 mmol/L EM. Potentials were recorded at second cochlear turn. In experiment depicted in B, anoxia was induced by both stopping respirator and terminating perilymphatic perfusion.

DISCUSSION

Since the first report by Mintz et al⁵ in 1973, there has been a growing literature on the ototoxicity of erythromycin.⁴ In spite of this fact, the mechanism by which erythromycin causes ototoxicity has still to be elucidated.

Stupp et al⁶ found considerable hair cell loss after intratympanic administration of erythromycin. Although their results showed potential ototoxicity of the drug, the route of application was different, so this cannot be regarded as the mechanism of the clinically observed hearing loss caused by intravenous erythromycin. Moreover, the hair cell loss in their study is inconsistent with the reversibility of the hearing loss we found.

Brummett⁴ reported a change of ABR in both guinea pigs and human patients by the intravenous administration of erythromycin lactobionate. He observed that erythromycin caused an increase in the latency of wave IV, followed in sequence by the other waves, in guinea pigs, and an increase in the latency of wave V (exclusively) in the patients. He concluded that erythromycin ototoxicity may be due to an effect on the central auditory pathways. This hypothesis is strengthened by several other observations of central nervous system effects in patients receiving erythromycin.⁴

However, the present data strongly suggest direct action of intravenously applied erythromycin on the inner ear, mainly on the stria vascularis, where the production of the EP is thought to take place.¹² This interpretation is strengthened by recent experiments in which erythromycin was shown to cause a decrease in the transepithelial potential of the stria marginal cells and of the homologous dark cells in the vestibular labyrinth.¹³ The decrease of the CM is not likely due to hair cell damage, but rather, may be a secondary effect due to the suppression of the EP. These observations of cochlear dysfunction are not in agreement with those of Brummett,⁴ but are in accordance with the observations made by Sacristan et al,⁷ who reported absence of waves I to III of the ABR during treatment with erythromycin, and suggested an impairment of the peripheral portion of the auditory pathway.

The primary action of erythromycin on the stria vascularis can account for the reported audiogram pattern of erythromycin ototoxicity, which is usually flat and uniform throughout all frequencies,⁴ being different from the high-frequency loss seen in aminoglycoside ototoxicity.

In the present study, the decrease in the cochlear potentials was noticed from an erythromycin dose of

100 mg/kg body weight of the guinea pig, which is not much different from the warning that clinical doses of erythromycin of more than 4 g/d may cause erythromycin ototoxicity.^{14,15} The reversibility of the EP and the CM found in this study are also in good accordance with the clinical picture of erythromycin ototoxicity. Our experiments showed that the effect of intravenous erythromycin is reversible up to 150 mg/kg. In addition, the negative component of the EP, which is thought to have an intimate relation with the integrity of the hair cell, was not affected after the same dose of intravenous erythromycin.

In contrast, perilymphatic perfusion of 3 mmol/L of erythromycin irreversibly affected both the EP and CM. Although perilymphatic perfusion needs further study with lower concentrations of erythromycin, the decrease of the CM was very drastic compared to the change of the EP. The CM started to decline sharply during the period when the EP was still near its original values, so we considered the drug to have exerted a potent action directly on the organ of Corti and/or the hair cells. Our preliminary histologic study revealed a degeneration of the hair cells after perfusion with 3 mmol/L erythromycin. The initial increase of the EP can probably be accounted for as a result of the decrease of the negative component of the EP, which is related to the integrity of the hair cells. Similar patterns of changes in the EP and CM were reported by Marcus et al¹⁶ with perilymphatic perfusion with vanadate, which has a dominant effect on the hair cells.

Complete reversibility of erythromycin ototoxicity is questioned because of the presence of rare clinical reports of incomplete recovery from hearing loss. Dylewski¹⁵ reported irreversible hearing loss in a 73-year-old woman who received 2 g/d of intravenous erythromycin lactobionate for 7 days. The reason for the occurrence of irreversible hearing loss was presumed by the author to be decreased elimination of the drug due to associated hepatic dysfunction caused by primary biliary cirrhosis. Although our experiments showed that the effect of intravenous erythromycin is reversible up to one dose of 150 mg/kg, we sometimes encountered an incomplete recovery of the CM when the dose of erythromycin was increased. Further study, including histologic examination, is planned on the reversibility of erythromycin ototoxicity, together with the different effects of intratympanic and perilymphatic application.

Although concomitant reversible dysfunction of the central auditory pathway cannot be excluded, this study reveals for the first time that the intravenous administration of erythromycin in the guinea pig can cause inner ear dysfunction at concentrations within the range of clinical dosage in humans.

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EFFECTS OF PLATELET ACTIVATING FACTOR ON VASCULAR PERMEABILITY OF THE MIDDLE EAR MUCOSA

CHUNG-KU RHEE, MD

CHEONAN, KOREA

YONG-SOO PARK, MD

SEOUL, KOREA

TIMOTHY T. K. JUNG, MD, PHD

LOMA LINDA, CALIFORNIA

STEVEN A. LONG, MD

LOMA LINDA, CALIFORNIA

DAVID DAVAMONY, MS, MPH

LOMA LINDA, CALIFORNIA

Platelet activating factor (PAF), a potent inflammatory mediator, seems to play a significant role in the pathogenesis of otitis media with effusion (OME), along with other inflammatory mediators such as leukotrienes and prostaglandins. The purpose of this study was to investigate the effect of PAF on the vascular permeability of middle ear mucosa, in an experimental OME model using chinchillas. We injected PAF in doses of 1, 4, 8, and 16 μ g and normal saline as a control into the bullae of chinchillas. Vascular permeability was measured by the Evans blue vital dye technique. All the PAF-injected animals showed a significant increase in middle ear vascular permeability compared to the control group. This study demonstrated that PAF in the middle ear cavity contributes significantly to the development of OME by increasing the vascular permeability of the middle ear mucosa.

KEY WORDS — otitis media with effusion, platelet activating factor, vascular permeability.

INTRODUCTION

Platelet activating factor (PAF), a biologically active phospholipid (1-*o*-alkyl-2-acetyl-sn-glyceryl-3-phosphoryl-choline), is a potent inflammatory mediator and has been shown to be involved in many pathologic processes, especially in inflammation and allergy.^{1,2} After appropriate stimulation, PAF is produced from various inflammatory cells such as neutrophils, platelets, eosinophils, macrophages, monocytes, mast cells, and vascular endothelial cells.^{1,2} In addition to promoting aggregation and degranulation of platelets, PAF induces an increase of vascular permeability (VP), chemotaxis of inflammatory cells, bronchoconstriction, an increase of bronchial hyperresponsiveness, an increase of airway mucous secretion, and initiation of hypotension, shock, and death.^{1,2} Platelet activating factor is a potent vasoactive agent and its effect on VP is 1,000-fold more potent than that of histamine.³ When injected into animal and human skin, PAF elicits wheal and flare responses due to the increase of VP.^{4,5} An intravenous injection of PAF induces an increase of VP in the lungs, trachea, bronchi, and pancreas.^{6,7} While PAF can be assumed to cause a similar increase of VP in the

middle ear (ME), this has not been previously demonstrated.

Platelet activating factor has been assayed from human middle ear effusion (MEE) and has been suggested to participate in the pathogenesis of otitis media with effusion (OME).^{8,9} When injected into bullae of chinchillas, PAF has induced OME.¹⁰ The histopathology of PAF-induced experimental OME showed edema, hyperemia, focal hemorrhage, and inflammatory cell infiltration, which are felt to be related to the effects of a PAF-induced increase of VP.¹⁰

We hypothesized that PAF in the ME induces an increase of VP of the ME mucosa. The purpose of this study is to investigate the effects of PAF on the VP of ME mucosa in experimental OME.

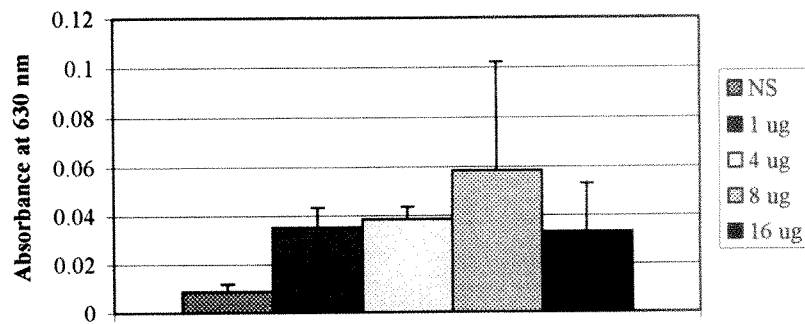
METHOD

We used the Evans blue vital dye technique in this study. Vital dyes readily form complexes with plasma albumin and accumulate in tissues. The degree of VP can be estimated by measuring the amount of dye

From the Department of Otolaryngology—Head and Neck Surgery, Dankook University College of Medicine, Cheonan (Rhee), and the Department of Otolaryngology, Catholic Medical College, Seoul (Park), Korea, and the Division of Otolaryngology—Head and Neck Surgery, Loma Linda University School of Medicine, Jerry L. Pettis Memorial Veterans Hospital, Loma Linda, California (Long, Jung, Davamony). This study was partially supported by grants from the Deafness Research Foundation, the Department of Surgery, Loma Linda University School of Medicine, and Jerry L. Pettis Memorial Veterans Hospital. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Loma Linda University School of Medicine.

CORRESPONDENCE — Chung-Ku Rhee, MD, Dept of Otolaryngology—Head and Neck Surgery, Dankook University College of Medicine, San-29 Anseo-Dong, Cheonan, Choongnam-Do, 339 714 Korea.

Absorbance of Evans blue dye at 630 nm. Absorbance is plotted for each chinchilla group. Key indicates amount of platelet activating factor injected into each ear. NS — normal saline.



extravasation. Previous studies have shown the Evans blue dye technique to be highly sensitive and well suited for the evaluation of VP in the ME.¹¹ Normal saline (NS) or different doses of PAF were injected into the ME of chinchillas through the superior bullae, by means of a tuberculin syringe, with a ventilation needle in place to prevent barotrauma to the bullae.

Induction of Otitis Media. All of the experiments were performed under ketamine hydrochloride (30 mg/kg) and xylazine hydrochloride (2 mg/kg) anesthesia. Twenty-eight healthy chinchillas were randomly assigned to five groups: the control group received 0.5 mL of NS in each superior bulla, and the four experimental groups each received either 1, 4, 8, or 16 µg of PAF in 0.5 mL of NS in each superior bulla. The control group and the groups receiving 1 and 4 µg each consisted of 4 animals, yielding 8 ears. The groups receiving 8 and 16 µg of PAF each consisted of 8 animals, yielding 16 ears. Twenty-four hours after injection, ears were examined by otomicroscopy and tympanometry. When a significant volume of MEE was present, it was aspirated from each bulla.

Evans Blue Vital Dye Technique. After the MEE was aspirated, Evans blue dye at a dose of 20 mg/mL per kilogram of body weight was injected into the femoral vein. Thirty minutes after dye injection, the vascular system was washed out by exsanguination and perfusion of 100 mL of NS through an intra-aortic catheter. Then, each ME cavity was lavaged with 0.5 mL of formamide for 5 minutes to collect the extravasated Evans blue. The absorbance of each collected sample was measured at a wavelength of 630 nm to determine the degree of dye extravasation by spectrophotometry. Adequate lavage fluid was not collected from 1 of the ears injected with 8 µg of PAF, leaving 15 ears, and fluid from 1 of the ears injected with 16 µg of PAF was contaminated with blood, again leaving 15 ears for spectrophotometry. The data were analyzed with the SPSS statistical package. Two-tailed Student's *t*-tests for independent variables and analysis of variance were utilized. Significance was determined at the *p* < .05 level.

RESULTS

Absorbance of Evans blue at 630 nm in each group was as follows (mean ± SD): control, 0.0089 ± 0.003 (*n* = 8); 1 µg, 0.0351 ± 0.008 (*p* = .009; *n* = 8); 4 µg, 0.0383 ± 0.005 (*p* < .001; *n* = 8); 8 µg, 0.0582 ± 0.044 (*p* = .001; *n* = 15); and 16 µg, 0.0330 ± 0.020 (*p* = .004; *n* = 15). The results show that the increase of VP in all of the PAF-injected groups compared to the NS-injected control group was statistically significant (see Figure). Duncan's multiple range testing also showed a significant difference between the 8- and 16-µg groups at *p* = .05.

DISCUSSION

There are several methods available to measure VP. Morphologic studies can be done to identify the sites of VP by injecting colloidal tracer particles, such as mercuric sulfide or carbon, into the vascular system. The tissues are then examined by either light or electron microscopy. This method is time-consuming and has the disadvantage of not measuring the dynamic aspect of VP. Vital dyes such as Evans blue, pontamine sky blue, and trypan blue readily form complexes with plasma albumin and accumulate in tissues. The degree of VP is estimated by measuring the amount of dye extravasation. The dye extraction method shows a high efficiency of dye recovery. As confirmed in our study, the vital dye technique appears to be a simple and convenient procedure for routine quantification of ME and cutaneous VP. The mechanism of increase of VP induced by PAF has been studied previously in endothelial cells; PAF has been shown to cause a reversible change of endothelial cell shape, and endothelial cells tend to loosen their contact with contiguous endothelial cells, resulting in increased permeability across the endothelial cell monolayer.^{12,13}

The present study measured VP in the ME of chinchillas. We have previously reported that PAF in doses of 1, 4, 8, or 16 µg injected into the ME cavity of chinchillas induced OME, which may be due to the increase of VP induced by PAF.¹⁰ The increase of VP induced by PAF appears to have contributed to the pathologic changes observed in our previous study of

PAF-induced experimental OME.¹⁰ Inflammatory mediators such as LTC₄, PGE₂, and PAF were released from inflammatory cells mobilized into the ME mucosa and ME cavity by the chemotactic activities of the locally injected PAF. A strong chemotactic property of PAF explains why higher levels of PAF were detected than were injected in the ME. It appears that the VP could have been increased directly by the injected PAF and indirectly by these newly released cyclooxygenase and lipoxygenase products¹⁴ and PAF. High-performance liquid chromatography assay has previously demonstrated high levels of LTC₄ and PAF and low but significant levels of PGE₂.¹⁰ According to our previous studies, PGE₂ does not seem to play as important a role as leukotriene or PAF in acute inflammation.^{10,15,16} In animal and human skin studies of PAF-induced inflammatory responses, PGE₂ alone did not increase VP, but PGE₂ admixed with PAF potentiated the VP induced by PAF.⁴ In another study that compared local responses on human skin between PAF and PGE₂, PAF was found to be 50 times more potent than PGE₂ on a molar basis in inducing a wheal.⁵ However, in both OME and an acute inflammatory response of the skin, the inflammatory response induced by PAF can be potentiated by inclusion of exogenous prostaglandins.^{4,15} As suggested by Williams,¹⁷ inflammation involves the release both of mediators important for

their permeability-increasing activity (such as PAF) and of vasodilating mediators (such as PGE₂). A synergistic interaction between PAF and PGE₂ in skin inflammation of guinea pigs and human volunteers has been reported.¹⁸ This synergistic interaction between PGE₂ and PAF must have been present in this experimental OME.

Previous studies have shown that a point may be reached where further increases in PAF may actually lead to decreased effects.¹⁰ Similar findings were seen in the present study, in that ears injected with 16 µg of PAF had significantly less VP than did ears injected with 8 µg. As of yet, no adequate explanation has been found for this phenomenon. It may be speculated that some sort of inhibitory feedback mechanism is induced by the higher concentrations of PAF.

CONCLUSION

This study demonstrated that local injection of PAF into the ME contributes to the development of OME — directly, by increasing VP of the ME mucosa, and probably indirectly, by releasing other inflammatory mediators such as leukotrienes and prostaglandins and increasing the concentrations of PAF. The increase of VP induced by PAF may be an important mechanism of production of MEE in otitis media.

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NEONATAL HEARING SCREENING

The European Consensus Development Conference on Neonatal Hearing Screening will be held in Milan, Italy, May 15-16, 1998. For more information, contact Dr F. Grandori, Centre of Biomedical Engineering, Polytechnic of Milan, Piazza Leonardo da Vinci 32, 20133 Milan, Italy; fax +39-2-23993360; email ecdc@elet.polimi.it.

IMAGING CASE STUDY OF THE MONTH

CONGENITAL CHOLESTEATOMA ISOLATED TO THE MASTOID

MICHAL LUNTZ, MD

TEL-AVIV, ISRAEL

FRED TELISCHI, MD

MIAMI, FLORIDA

BRADFORD RESS, MD

MIAMI, FLORIDA

BRIAN BOWEN, PhD, MD

MIAMI, FLORIDA

THOMAS BALKANY, MD

MIAMI, FLORIDA

Congenital cholesteatoma may originate in various locations within the temporal bone, including the petrous apex, the area of the geniculate ganglion, the jugular foramen, the middle ear, the mastoid process, and the squamous portion of the temporal bone. According to Nager,¹ the mastoid process is the least frequent. We report an unusual case of congenital cholesteatoma that originated in and was restricted to the mastoid process and presented as an expansile mass that compressed the cerebellum.

CASE REPORT

A 54-year-old woman presented at the University of Miami Ear Institute in 1993 with right neck pain and persistent instability. She had no previous ear surgery. The findings on physical examination were unremarkable, and a hearing test showed a symmetric sloping high-tone sensorineural hearing loss.

High-resolution computed tomography (CT) of the temporal bones revealed an expansile lytic lesion in the right mastoid (Fig 1). Her mastoid pneumatization was well developed bilaterally. The middle ear, inner ear, attic, aditus ad antrum, and antrum were within normal limits. Magnetic resonance imaging (MRI) of the brain revealed a nonenhancing expansile lesion of the right mastoid, involving the mastoid tip and the posterior aspect of the mastoid air cells (Fig 2). The lesion had compressed the adjacent right cerebellar hemisphere and caused narrowing of the right transverse sinus. On T2-weighted images it was hyperintense, and on T1-weighted images, moderately hypointense. The signal characteristics were those of a semisolid mass, not of a simple cyst.

The patient was lost to follow-up until 1 year later, when she presented with similar symptoms. A subsequent CT without contrast showed no significant differences from the original findings.

At surgery the mastoid was found to be filled by the cholesteatoma, which had almost completely destroyed the tegmen mastoideum, the posterior fossa bony plate, and the bony plate covering the sigmoid sinus. The sinodural angle had been invaded and the superior petrosal sinus had been exposed. However,



Fig 1. Erosion and expansion of right mastoid. Axial computed tomogram (1-mm-thick section, with gray scale adjusted for bone imaging) at level of epitympanum and internal auditory canal demonstrates erosion and expansion of mastoid, especially at medial margin (arrows), as well as destruction of normal bony septations between mastoid air cells. Internal auditory canal, bony labyrinth, and tympanic cavity are normal.

From the Department of Otolaryngology, Jackson Memorial Hospital and the University of Miami Ear Institute (Luntz, Telischi, Ress, Balkany), and the Department of Radiology, Section of Neuroradiology, University of Miami School of Medicine (Bowen), Miami, Florida. Dr Luntz is currently in the Department of Biomedical Engineering, Tel-Aviv, Israel.

CORRESPONDENCE — Fred Telischi, MD, Dept of Otolaryngology, Jackson Memorial Hospital and the University of Miami Ear Institute, PO Box 016960, Miami, FL 33130.

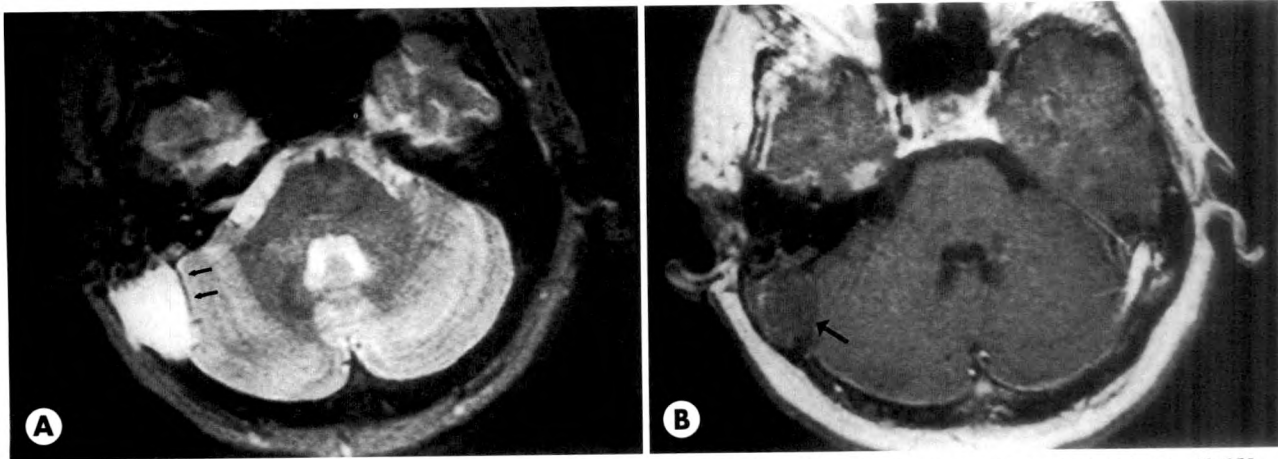


Fig 2. Expansile mass in right mastoid, compressing cerebellum. A) Axial, T2-weighted magnetic resonance image (2,650 milliseconds [ms]/80 ms/1 [TR/TE/excitations]), at same level as computed tomographic image, shows hyperintense mass with signal intensity similar to that of cerebrospinal fluid. Low-signal border laterally represents bony margin seen in Fig 1. Medial border represents dura and eroded bone (arrows). Medial margin compresses right cerebellar hemisphere. B) Axial, T1-weighted image (600 ms/30 ms/1), obtained after administration of intravenous gadolinium, shows fairly uniform, nonenhancing, hypointense mass (arrow) with signal intensity intermediate between those of cerebrospinal fluid and gray matter.

Korner's septum was intact, with no expansion or invasion of the cholesteatoma anterior to it. The middle ear, the attic, the aditus ad antrum, and the antrum were unaffected. The cholesteatoma was completely removed through an intact canal wall procedure. Nine months after the operation the patient's symptoms had disappeared, and the patient refused a second-look procedure.

DISCUSSION

Congenital cholesteatomas of the temporal bone can be classified according to their anatomic origin. The clinical presentation and imaging characteristics are determined by their location. To our knowledge, only two other reports have been published on the specific clinical presentation of congenital cholesteatoma originating in the mastoid: one of them by Derlacki and Clemis² in 1965, and the other by Nager³ in 1982. In the latter case the mastoidal origin of the cholesteatoma was presumed, but could not be verified, since by the time of diagnosis the cholesteatoma had occupied the whole middle ear cleft, including the middle ear, and invaded the intracranial space through the middle fossa dura. Thus, in characterizing congenital cholesteatoma of the mastoid, we can only refer to Derlacki and Clemis' patient and our own patient, as these are the only known descriptions of congenital cholesteatoma of the mastoid with no middle ear involvement. Both patients were adults whose initial complaint was pain over the mastoid area and the upper neck. Although the patient in the earlier study had conductive hearing loss, it was found at surgery that the ossicular disease was unrelated to and had no anatomic continuity with the cholesteatoma in the mastoid. Thus, as one would

expect (since the middle ear is not involved in the cholesteatomatous process), conductive hearing loss is not part of the clinical syndrome.

The mastoid air cell system was found to be well developed in our case, as is usually found in congenital cholesteatoma.^{4,5} Derlacki and Clemis² did not relate the size of the mastoid in their case. Both patients presented with what appeared to be vestibular symptoms. While those of Derlacki and Clemis' patient were related to erosion of the bony labyrinth, in the present case the sensation of continuous disequilibrium was probably attributable to compression by the lesion on the cerebellar hemisphere.

Imaging (mastoid X-ray) in the first patient revealed a translucent cyst in the mastoid, which had grown from 1948 to 1955.² In the present patient, CT and MRI scans showed an expansile lytic lesion with features of a complex cystic mass in the mastoid, with no significant changes over a 1-year period. Diagnosis was assisted by the presence of MRI characteristics of cholesteatoma, namely, hypointensity on T1- and hyperintensity on T2-weighted images⁶ (Figs 1 and 2).

At surgery, our patient and that of Derlacki and Clemis² showed extensive erosion of the bony plate covering the middle fossa dura and the sigmoid sinus. In our patient, the bony plates covering the sigmoid sinus and the posterior fossa dura were also extensively eroded.

In summary, the characteristic features of congenital cholesteatoma originating in the mastoid process would appear to be the following.

1. Initial presentation as neck pain.
2. Computed tomographic findings of a cystic,

expansile lesion occupying the mastoid process without involving the middle ear. Due to its space-occupying effect, it may compress the cerebellum and the dural sinuses adjacent to it and/or erode the bony labyrinth.

3. Magnetic resonance findings of hyperintensity on T2-weighted images, with little or no peripheral enhancement on postcontrast T1-weighted images, typical of congenital cholesteatoma involving the petrous apex.⁶

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CLINICOPATHOLOGICAL CONSULTATION

HUMAN IMMUNODEFICIENCY VIRUS-1 INFECTION OF THE LYMPHOID TISSUES OF WALDEYER'S RING

SARAH S. FRANKEL, MD

WASHINGTON, DC

BRUCE M. WENIG, MD

WASHINGTON, DC

ALFIO FERLITO, MD

PADUA, ITALY

Human immunodeficiency virus-1 (HIV-1) infection is a fatal retroviral infection that may first present clinically as enlargement of the lymphoid tissues of Waldeyer's ring. These tissues are a major site of viral replication. The presence of the virus in these tissues causes a unique constellation of diagnostic histopathologic features, including florid follicular hyperplasia, follicle lysis, and productively HIV-1-infected multinucleated giant cells of probable dendritic cell origin. Serologic evaluation is confirmatory of HIV infection. With the recent advances in antiretroviral chemotherapy, the early institution of which may significantly prolong life and disease-free interval, the recognition of the clinical and pathologic parameters of HIV-related enlargement of Waldeyer's ring tissues is essential.

INTRODUCTION

Infection with the human immunodeficiency virus (HIV) leads to a chain of events within the host immune system that ultimately results in immunosuppression and the development of acquired immunodeficiency syndrome (AIDS). The critical pathogenic event in HIV infection is the destruction of CD4+ (helper) T-cell lymphocytes and the resulting loss of cellular immunity.^{1,2} Initially, presentation of HIV to lymphoid tissues via blood mononuclear cells and dendritic cells results in extensive reactive lymphoid proliferation that causes enlargement of the infected lymph nodes and extranodal lymphoid tissues in the early and subclinical stages of disease.³⁻¹⁰ Primary HIV-1 infection results in a spectrum of histopathologic changes that may represent the initial manifestation of HIV infection in otherwise asymptomatic patients. The clinical enlargement of tonsillar and particularly nasopharyngeal lymphoid tissue (adenoids) may represent the earliest clinical manifestation of HIV-1.¹⁰

CLINICAL FEATURES

The clinical features of tonsillar and adenoidal disease in HIV patients are extensively detailed in other reports.⁷⁻¹⁰ In brief, HIV-induced tonsillar and adenoidal enlargement is more common in men than women, and occurs most frequently in the third to

fifth decades of life (median age, fourth decade). The clinical presentations vary and include nasal congestion, airway obstruction, sore throat (pharyngitis), otitis media unresponsive to antibiotic therapy, otalgia, facial weakness, fever, and a nasopharyngeal or tonsillar mass.⁷⁻¹⁰ Clinically, the tonsils and adenoids are enlarged, usually bilaterally, but unilateral enlargement may occur. The enlargement of the tonsils or adenoids may raise the concern of a hematomatous lymphoid or epithelial neoplasm, prompting surgical removal of the enlarged organ. Concurrent (unilateral) cervical adenopathy may be present.^{8,10} Large ulcers leading to complete destruction of the tonsil have also been reported; no causative agents could be identified by histopathologic, microbiologic, and laboratory tests.¹¹ It is difficult to determine whether these lesions are a type of giant aphthous ulcer or a malady unique to tonsils in patients with AIDS.¹¹ Wenig et al¹⁰ report that of their entire group of 12 patients, 4 were known or suspected at the time of presentation to be infected with HIV and/or suffering from AIDS. The 4 patients who were known or suspected to be HIV-infected tested positive for HIV by serologic evaluation, but were without evidence of opportunistic infections. The remaining 8 patients were not known to be HIV-infected and were not known to be in any of the risk groups associated with HIV infection. Other than enlarged tonsils or adenoids, these patients presented without any of the

From the AIDS Division of the Department of Infectious and Parasitic Disease Pathology (Frankel) and the Department of Otolaryngic and Endocrine Pathology (Wenig), Armed Forces Institute of Pathology, Washington, DC, and the Department of Otolaryngology, University of Padua, Padua, Italy (Ferlito). The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Navy, the Department of the Army, or the Department of Defense.

CORRESPONDENCE — Bruce M. Wenig, MD, Dept of Otolaryngic and Endocrine Pathology, Armed Forces Institute of Pathology, Building 54, Room 2025, Washington, DC 20306-6000.

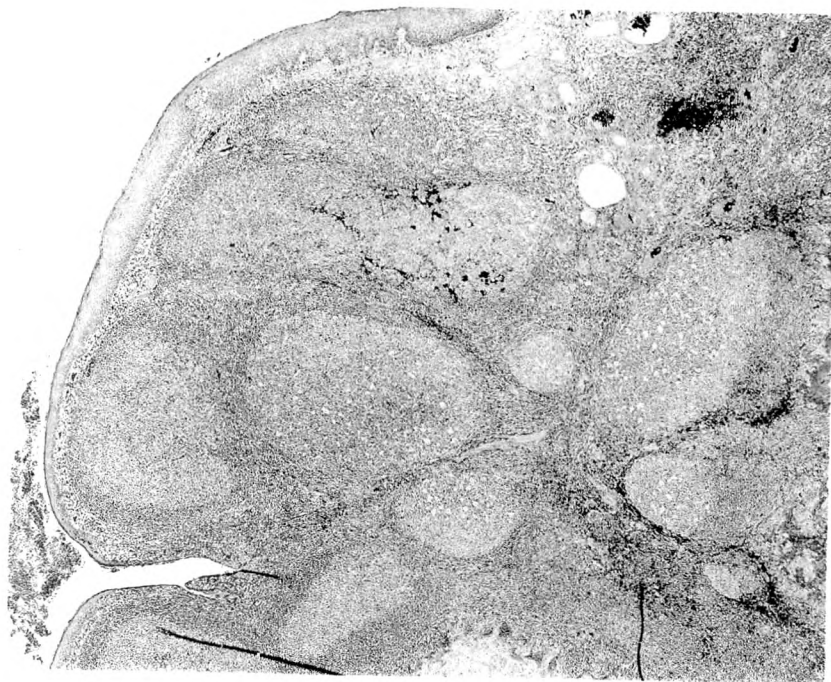


Fig 1. Early histologic manifestations of HIV infection included presence of florid follicular hyperplasia, characterized by enlarged and irregularly shaped germinal centers, some approximating surface epithelium, with attenuation or absence of mantle cell lymphocytes (H & E, original $\times 30$).

clinical stigmata of HIV infection. The risk factors for the patients who were known or suspected to be HIV-positive included homosexuality, blood transfusions, and/or intravenous drug abuse.

HISTOLOGY

The histomorphologic changes in HIV-induced tonsillar and adenoidal enlargement vary with the progression of disease. In the early stages of infection, the histomorphology may include florid follicular hyperplasia (Fig 1) with and without follicular fragmentation, and follicle lysis with areas of follicular involution (Fig 2). Additional findings included the presence of monocytoid B-cell hyperplasia, paracortical and interfollicular zone expansion with immunoblasts and plasma cells, interfollicular clusters of high endothelial venules, intrafollicular hemorrhage, and the presence of multinucleated giant cells (MGCs). The giant cells characteristically cluster adjacent to or within the adenoidal surface epithelium or the tonsillar crypt epithelium (Fig 3).¹⁰

The histologic features in patients with more advanced stages of disease contrast with those described above and correlate with the lymphoid obliteration seen in the terminal stages of HIV infection or AIDS.⁶ In these cases, there is effacement of nodal architecture, loss of the normal lymphoid cell population with replacement by a benign plasma cell infiltrate, and the presence of increased vascularity. The MGCs characteristically seen in the early and chronic stages of disease are not identified in the more advanced stages of HIV infection. Wenig et al¹⁰ report that special stains for microorganisms (other

than HIV) were negative in all of their cases studied.

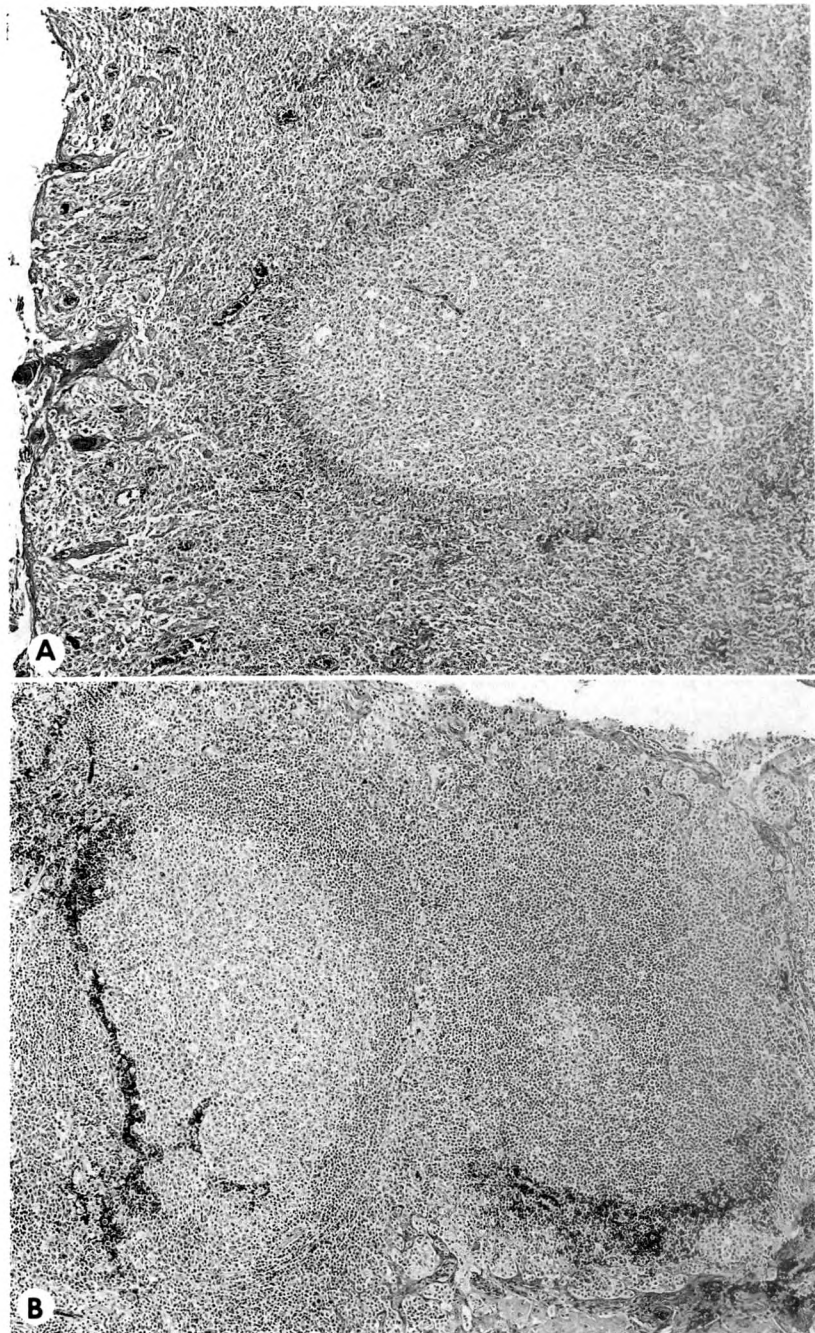
IMMUNOHISTOCHEMISTRY AND IN SITU HYBRIDIZATION

Reactivity for HIV p24 (gag protein), an indicator of active HIV infection,^{10,12} is consistently identified in the early and chronic stages of disease.¹⁰ Anti-HIV p24 reactivity is seen within the follicular dendritic cell (FDC) network of the germinal centers, in scattered interfollicular lymphocytes, in the MGCs, and within intraepithelial cells of crypt epithelium (Fig 4).¹⁰ The HIV p24-positive intraepithelial cells are positive for S-100 protein (a dendritic cell marker), and their morphologic appearance correlates with the appearance of dendritic cells (DCs).^{10,13} Reactivity with both B-cell (CD20) and T-cell markers or subsets (CD45RO, CD3, OPD4) is seen within the germinal centers and in the interfollicular regions, as well as in scattered intraepithelial cells.^{10,13}

The patients in more advanced stages of disease, characterized by loss of germinal centers and the presence of a predominant plasma cell infiltrate, show a relative absence of lymphoid cell markers (CD45RB, CD3, or OPD4). In these cases, the plasma cells show reactivity with κ and λ light chains indicative of a benign proliferation. Surface and crypt epithelia are cytokeratin-reactive. Immunoreactivity with Epstein-Barr virus latent membrane protein, herpes simplex virus, or cytomegalovirus is not present.¹⁰

Evidence of HIV RNA by in situ hybridization is seen in the FDC network, in the MGCs, and in mature lymphocytes localized to the germinal centers, to

Fig 2. Histologic changes suggesting early HIV infection include following. A) Disruption of germinal centers by "infiltrating" small lymphocytes, creating moth-eaten appearance referred to as follicle lysis (H & E, original $\times 30$). B) Follicular involution (to right of center), characterized by small-sized follicles with disappearance of tingible body macrophages and absence of clearly defined mantle zones (H & E, original $\times 75$). Separate follicle (to left of center) shows follicular hyperplasia.



interfollicular zones, and within the surface and/or crypt epithelia (Fig 5).¹⁰ The strongest signal is present in the MGCs.

DISCUSSION

Human immunodeficiency virus-1 infects peripheral blood cells, including lymphocytes,^{14,15} monocytes or macrophages,¹⁶⁻¹⁸ and DCs,¹⁹ with subsequent hematogenous distribution of the virus throughout the body. The prime target of the virus is the lymphoid tissues. Following HIV infection, the infected peripheral lymphocytes, monocytes or macro-

phages, and DCs are believed to carry the virus, infecting lymph nodes throughout the body, leading to a series of irreversible morphologic changes. The morphologic changes seen in affected lymph nodes and peripheral lymphoid tissues represent a continuum that varies according to the duration and progression of disease.⁶ The HIV-associated morphologic changes are not restricted to nodal lymphoid tissues, but also affect extranodal lymphoid tissues such as Waldeyer's tissue as well as the thymus.^{7-10,20} The histopathologic changes in the adenoids and tonsils depicted in this study are identical to those described in the lymph nodes of patients

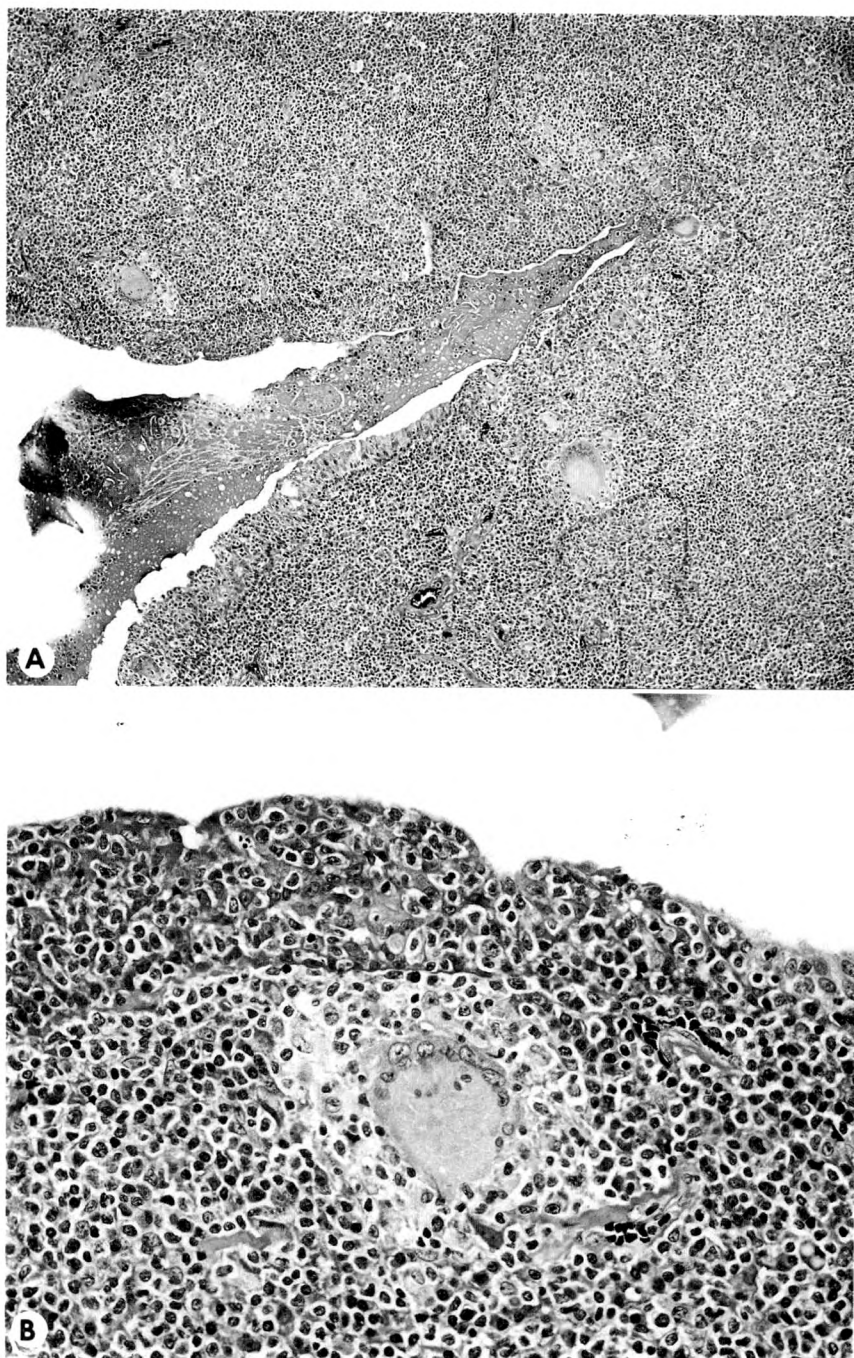


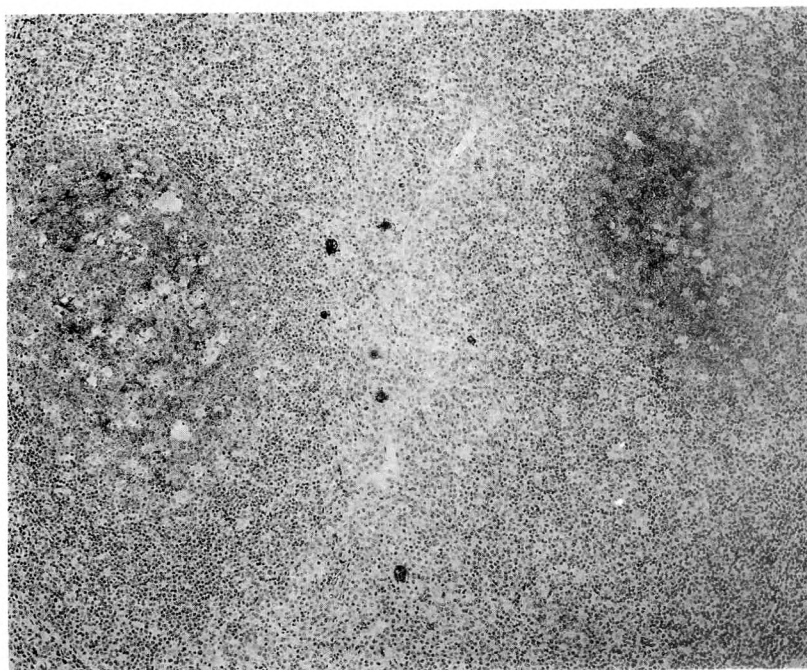
Fig 3. Multinucleated giant cells were specific for HIV infection. A) Giant cells characteristically localized to crypt epithelium or subepithelial area (H & E, original $\times 75$). B) Higher magnification, illustrating multinucleated giant cell approximating nasopharyngeal lymphoepithelium (H & E, original $\times 300$).

infected with HIV.^{3,4,6,21} In conjunction with the spectrum of changes of the germinal centers and interfollicular regions, the presence of MGCs in the tonsillar and adenoidal tissue is a key diagnostic feature.^{10,13}

Wenig et al¹⁰ and Frankel et al¹³ posed the question whether the MGCs are pathognomonic for tonsillar or adenoidal HIV infection. Wenig et al¹⁰ showed that the MGCs were readily apparent in all of the patients in the early and chronic stages of HIV infection, but were absent in those patients who were in more advanced stages of disease (cases 2 and 4). Of

interest is the fact that 3 cases mentioned by Wenig et al¹⁰ showed light microscopic features in enlarged tonsils or adenoids that suggested the possibility of early HIV infection. In these cases, MGCs were not found and follow-up serologic studies were negative for HIV infection. Extensive follow-up failed to identify a specific cause for the enlarged tonsils and adenoids in these patients, who remain clinically well. While the morphologic changes associated with HIV lymphadenopathy are reproducible and have engendered histopathologic grading schemes felt to be prognostically significant,^{3,22-26} both the specificity of the light microscopic changes and the validity

Fig 4. Immunohistochemical reactivity included HIV p24 core antigen reactivity within follicular dendritic cells (left and right of center) and multinucleated giant cells (center area) (immunohistochemical stain for p24 gag protein, original $\times 75$).



of these changes as prognostic indicators have been questioned.^{26,27} In our experience, if MGCs are added to the other histologic features, then a diagnosis of HIV infection becomes highly suspicious but *always*

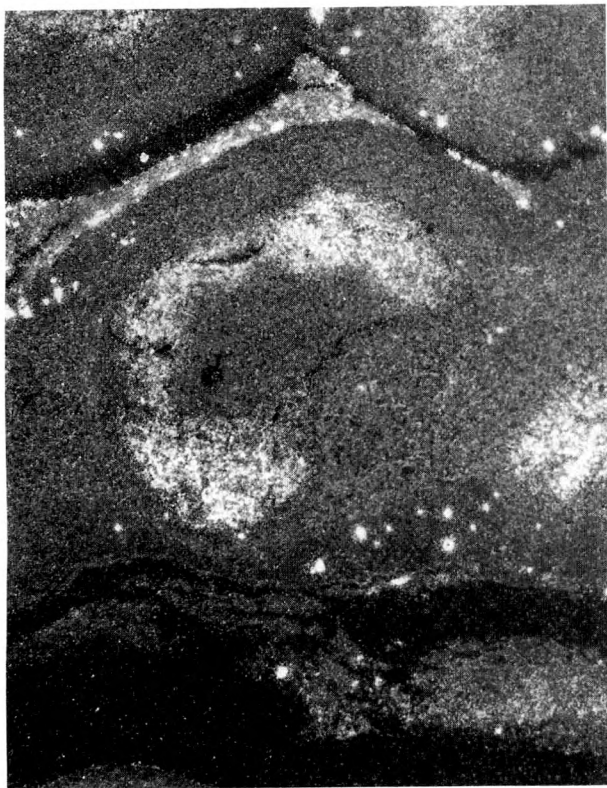


Fig 5. In situ hybridization for HIV-1 RNA using antisense riboprobe, dark-field microscopy, showing signal in several enlarged and irregularly shaped or fused germinal centers, as well as in scattered interfollicular cells and multinucleated giant cells, latter approximating epithelial layer (original $\times 25$).

requires confirmation by serologic studies. In the study by Wenig et al,¹⁰ 7 of 8 patients with histologic alterations suggesting HIV infection, but who were not known to be nor suspected of being HIV-positive, were HIV-positive by serologic (enzyme-linked immunosorbent assay) studies. The remaining patient refused serologic testing. Documented cases of HIV-infected patients have been reported in which MGCs were absent.^{8,28-30} However, we believe that finding florid follicular hyperplasia (with or without follicle lysis) together with MGCs is virtually diagnostic for HIV infection. Confirmation by immunohistochemical, in situ hybridization, serologic, or viral burden testing for HIV-1 is always required for definitive diagnosis.

The clinical spectrum of HIV infection includes three phases: early or acute, chronic, and crisis or final.³¹ The morphologic changes described above parallel the course of HIV infection. In the early stages of disease, the florid follicular hyperplasia of lymphoid tissues results in enlargement of the affected sites. This not only causes lymphadenopathy, but results in enlarged adenoids and/or tonsils that may cause airway obstruction and/or otitis media and raises the clinical concern for neoplastic involvement of these sites.⁸ Similar to the lymphoid tissue changes that occur with the progression of disease, the identification of the HIV also changes over time. In nodal tissues, the FDCs of the germinal centers have been shown to be reservoirs of HIV RNA.³²⁻³⁴ The FDCs entrap but do not actively produce HIV, allowing for presentation of the virus to competent immune cells of B-cell lineage.³¹ With progression of disease and continued immune suppression, the germinal centers

involute and then disappear.

The two types of DCs include FDCs found in germinal centers, which are mesenchymal-derived, and the bone marrow-derived DCs.³⁵ The FDCs retain native antigens as immune complexes on their cell surface and present native antigen to B cells. The bone marrow-derived DCs, referred to as interdigitating dendritic cells (IDCs), migrate from blood or afferent lymph to the T-dependent regions of peripheral lymphoid tissues.³⁵ The IDCs process antigens into immunogenic peptides and present these to T cells.³⁶ Hart and McKenzie³⁷ isolated and phenotypically characterized human tonsillar DCs and showed these cells to belong to the hematopoietic cell lineage of DCs. In addition to T helper cells and monocytes or macrophages, DCs also are infected by HIV.³⁸⁻⁴¹ Our findings confirmed the presence of HIV extracellularly as retained virus in the FDC network of adenoidal and tonsillar germinal centers. Consistently intense anti-HIV p24 core antigen reactivity was identified within the germinal centers, and in situ hybridization studies showed concentration of signal in the FDC network. As shown by Fox et al,³³ with advancing disease this reservoir disappears as the germinal centers involute. In addition to the FDCs, another pool of virus was identified within (intracellular) mucosal and submucosal DC syncytia (MGCs) and scattered lymphocytes (Fig 4).

During infections with HIV-1 of DC-T lymphocyte cell mixtures in culture, syncytial cells form and are the principal site in which viral p24 antigen and budding virions are found.⁴² In vitro, the syncytia exhibit immunohistochemical staining with both S-100 protein and p55, a marker of DC activation and T-cell antibodies. Though we have not been able to demonstrate immunoreactivity with markers for T-cell lymphocytes in tissue sections, we hypothesize that the MGCs represent an in vivo correlate for the

in vitro site of productive HIV-1 infection.

Transmucosal HIV-1 infection plays a critical role in sexual transmission,⁴³ and HIV-1 has been identified within mucosal cells of the cervix⁴⁴ and the gastrointestinal tract.⁴⁵⁻⁴⁸ Recent studies with simian immunodeficiency virus (SIV) in macaques have demonstrated clearly that adult macaques can be readily infected with SIV introduced nontraumatically in the oral cavity.⁴⁹ The mucosa of the tonsil and adenoid has been postulated as the likely route of viral entry.⁴⁹ This mechanism has also been suggested to play an important role in perinatal maternal-infant transmission, as well as in oral-genital transmission.

SUMMARY

The initial clinical presentation of HIV-infected individuals may include diseases of the upper aerodigestive tract. Infection with HIV may result in clinically enlarged adenoids or tonsils that may be suspicious for a neoplasm. On the basis of the morphologic changes, HIV infection can be suspected even in asymptomatic patients. Immunohistochemical, in situ hybridization, and serologic evaluations confirm the presence of HIV infection. Consistent and reproducible histologic features in the early and/or subacute stages of disease are seen, including florid follicular hyperplasia, follicle lysis, and MGCs. The MGCs preferentially localize to surface or crypt stratified squamous epithelium and are typically absent in patients who are in the advanced stages of disease. The MGCs may represent syncytia of infected T lymphocytes and activated DCs.¹¹ These changes may be the presenting signs of HIV-1 infection in otherwise asymptomatic individuals. With the advent of effective early antiretroviral chemotherapy, specifically, protease inhibitors, the importance of identifying early-stage HIV-1 infection has increased.

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ROLE OF ADENOIDS IN THE PATHOGENESIS OF OTITIS MEDIA: A BACTERIOLOGIC AND IMMUNOHISTOCHEMICAL ANALYSIS

THOMAS E. LINDER, MD

ZÜRICH, SWITZERLAND

HANS-PETER MARDER, PHD

LUCERNE, SWITZERLAND

JÜRIG MUNZINGER, MD

LUCERNE, SWITZERLAND

Adenoidectomy is frequently performed in children suffering from recurrent or chronic otitis media with effusion and is thought to produce a long-term effect in preventing further episodes of otitis media. Bacteriologic analysis of adenoids from 60 patients revealed a significantly elevated colonization rate of middle ear pathogens in children with a present or previous history of ear disease compared to children with adenoidal hypertrophy only. The predominant pathogen was nontypeable *Haemophilus influenzae*, followed by *Streptococcus pneumoniae* and *Moraxella catarrhalis*. Quantitative analysis did not demonstrate a bacterial overload in the otitis group. Lectin histochemical analysis of the adenoids revealed no significant differences between the three groups; nevertheless, colonization with *S pneumoniae* demonstrated an increased labeling pattern with succinylated wheat germ agglutinin, indicating the exposure of N-acetyl-glucosamine as part of its own receptor structure. On the basis of these results, we support the concept of adenoidectomy in order to remove a bacterial focus; however, we could not verify the hypothesis of bacterial overgrowth in the nasopharynx.

KEY WORDS — adenoidectomy, lectin, nasopharyngeal colonization, otitis media with effusion.

INTRODUCTION

Adenoidectomy and ventilation tube insertions are the most common surgical procedures performed in children. The placement of ventilation tubes immediately improves the patient's hearing, adequately ventilates the middle ear cleft, and has documented efficacy in patients with recurrent acute otitis media (rAOM) and chronic otitis media with effusion (cOME).¹ Adenoidectomy, however, is thought to produce a long-term effect in preventing further episodes of otitis media (OM).^{2,3} Most physicians tell their patients that adenoidectomy is meant to improve the ventilatory function of the eustachian tube (ET). Yet, adenoid enlargement is frequently detected in young children, and conflicting reports have failed to document a mechanical obstruction at the pharyngeal orifice. The strong correlation between nasopharyngeal and middle ear cultures in children with acute otitis media (AOM) implicates the adenoids as the source of infection. Potential overgrowth of pathogenic organisms may predispose the child to recurrent middle ear infections. Adenoidectomy is therefore intended to remove the origin of chronic or recurrent bacterial infection in a child with a dysfunctional ET.

The present study was undertaken to quantitatively determine the nasopharyngeal colonization rate of middle ear pathogens and to analyze the carbohy-

drate receptor structure of the adenoidal cell surface in children with and without a history of ear disease.

METHODS AND MATERIALS

All children seen at the outpatient clinic of the Department of Otolaryngology, Kantonsspital Luzern, and scheduled for adenoidectomy between August 1994 and January 1995 were continuously enrolled in our study. A thorough history was obtained, and otoscopy under the microscope, a hearing test, and tympanometry were performed prior to surgery. Sixty children, ranging in age from 2 to 12 years, were divided into three categories (Table 1). Group 1 consisted of patients with a history of recurrent otitis media with effusion (rOME) or cOME and middle ear effusion present at the time of surgery, group 2 were patients with a history of recurrent or chronic OM without effusion at myringotomy, and group 3

TABLE 1. DEMOGRAPHICS

	Group 1 (rOME and cOME)	Group 2 (OM)	Group 3 (Controls)
Number	27	12	21
Median age (y)	4.8	5	6.4
Age range (y)	2.1 - 9.1	1.7 - 7.8	3.2 - 12
Boys	17	8	14
Girls	10	4	7

rOME — recurrent otitis media with effusion, cOME — chronic otitis media with effusion, OM — recurrent or chronic otitis media without effusion at myringotomy.

From the Department of Otolaryngology (Linder) and the Institute for Medical Microbiology (Marder, Munzinger), Kantonsspital Luzern, Lucerne, Switzerland.

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CORRESPONDENCE — Thomas E. Linder, MD, Dept of Otorhinolaryngology, University of Zürich, Frauenklinikstr 24, 8091 Zürich, Switzerland.

TABLE 2. QUANTITATIVE OCCURRENCE OF MIDDLE EAR PATHOGENS IN NASOPHARYNX

Bacteria	Group 1 (n = 27)	Group 2 (n = 12)	Group 3 (n = 21)	p
Nontypeable <i>Haemophilus influenzae</i>	82% 5.5 ± 6.3	66% 2.7 ± 2.9	57% 3.7 ± 4.8	1 vs 3 p < .05 2 vs 3 NS 1 + 2 vs 3 NS
<i>Streptococcus pneumoniae</i>	26% 5.5 ± 6.9	42% 0.6 ± 0.6	19% 3.1 ± 3.4	NS
<i>Moraxella catarrhalis</i>	22% 7.2 ± 16.1	66% 1.9 ± 1.5	28% 5.8 ± 7.6	2 vs 3 p < .05 2 vs 1 p < .01 1 vs 3 NS 1 + 2 vs 3 NS

Data are percentages of patients (adenoids) with colonization, followed by means ± SD of colony-forming units (times 10⁵) per gram of adenoid.
NS — not significant.

included patients with adenoidal enlargement with suspected sleep apnea syndrome or patients with chronic tonsillitis of the palatine tonsils but no previous ear disease. Recurrent OM was defined as three or more episodes of AOM or otitis media with effusion (OME) within the last 12 months (verified by the pediatrician or private otolaryngology specialist), and cOME was diagnosed when the effusion lasted for longer than 3 months. No children had taken any antibiotics for at least 2 weeks prior to surgery, and none had craniofacial or suspected immunologic abnormalities. They all underwent adenoidectomy and myringotomy if middle ear effusion was suspected.

The adenoidal tissue was immediately transferred to the laboratory and separated for quantitative bacteriologic analysis, as well as tissue fixation with glutaraldehyde. The tissue specimens were weighed, manually crushed and homogenized, and inoculated onto sheep's blood agar and chocolate agar at standardized dilutions. Pathologic organisms were identified according to basic laboratory techniques and counted, and the number of colony-forming units (cfu) per gram of adenoidal tissue was calculated. To test whether or not the quantitative bacteriology counts varied across the three groups, we used the χ^2 test and an analysis of variance for unequal n-size (ANOVA). The coauthors responsible for the bacteriologic analysis were blinded as to the study group.

Considering the weight of the specimens and the dilution factors, we were able to detect bacterial

TABLE 3. MIDDLE EAR PATHOGENS VERSUS NORMAL FLORA

	Group 1	Group 2	Group 3
Middle ear pathogen*	85%	92%	66%
Normal flora only*	15%	8%	34%
Multiple pathogens	40%	58%	33%

Data are percentages of patients (adenoids).

*For groups 1 plus 2 combined versus group 3, p < .05.

counts starting at 500 cfu/g of adenoidal tissue. We specifically looked at the possible middle ear pathogens such as nontypeable *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis*. *Staphylococcus aureus* and group A streptococci were considered separately as potential pathogens.

The fixed tissue specimens were embedded in Epon and processed for lectin histochemical analysis with five different biotinylated lectins. Lectins are sugar binding proteins or glycoproteins of nonimmune origin with a high sensitivity for specific carbohydrate residues, and the labeling was performed by means of the avidin-biotin complex labeling technique providing a red color change of the chromagen. The technique has been published previously.⁴

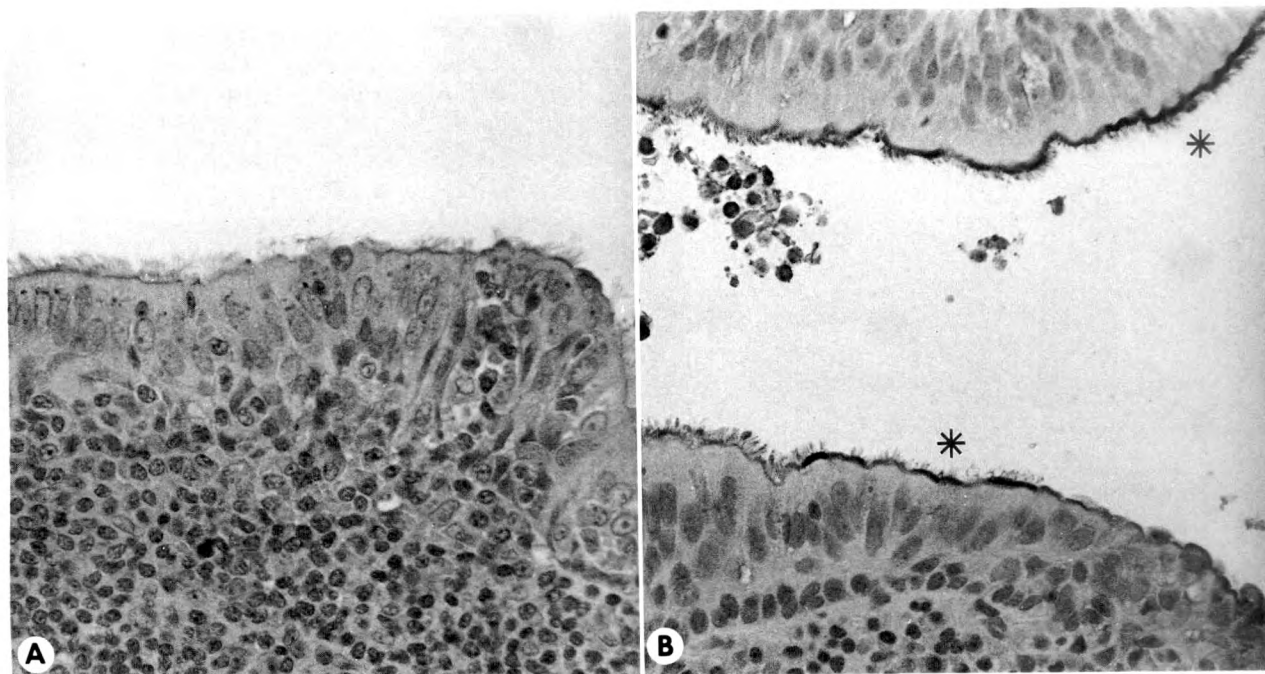
RESULTS

Eighty-two percent of adenoids of group 1 were colonized with a mean concentration of 5.5×10^5 cfu of nontypeable *H influenzae* per gram of adenoidal tissue (Table 2). Group 2 revealed 66%, and group 3, 57% colonization with nontypeable *H influenzae*. By

TABLE 4. LECTIN LABELING PATTERN

Lectin	Specificity	Group 1	Group 2	Group 3
SNA	NeuAc(α 2-6)Gal	+++	+++	+++
WGA	NeuAc GlcNAc(β 1-4)	+++	+++	+++
Succ				
WGA	GlcNAc(β 1-4)	48%++	30%++	35%++
BSL II	GlcNAc(α / β 1-4)	12%+	20%+	11%+
PNA	Gal(β 1-4)GlcNAc Gal(β 1-3)GalNAc	12%+	10%+	11%+

SNA — *Sambucus nigra* agglutinin, WGA — wheat germ agglutinin, Succ — succinylated, BSL II — *Bandeiraea simplicifolia* lectin II, PNA — peanut agglutinin, NeuAc — neuraminic acid, Gal — galactose, GlcNAc — N-acetyl-glucosamine, GalNAc — N-acetyl-galactosamine. For staining intensity: +++ — strong, ++ — moderate, + — weak. Percentages are of adenoids with positive staining.



Lectin labeling with succinylated wheat germ agglutinin. A) No staining of adenoidal cell surface. (Patient of group 3, 7×10^4 cfu/g nontypeable *Haemophilus influenzae*; 2.8×10^5 cfu/g *Streptococcus* type A.) B) Positive staining (asterisks) of adenoidal cell surface. (Patient of group 1, 9.6×10^5 cfu/g *Streptococcus pneumoniae*; 9.6×10^5 cfu/g nontypeable *H influenzae*.)

the χ^2 test, the differences between groups 1 and 3 reached statistical significance at $p < .05$. Group 2 and the combined groups 1 and 2 did not differ statistically from group 3. For *S pneumoniae*, the colonization rates for groups 1 through 3 were 26%, 42%, and 19%, respectively. There were no statistical differences between the three groups. It was interesting to note that the colonization rate for *M catarrhalis* was very similar between groups 1 and 3, but significantly different for group 2. However, the concentrations of bacterial pathogens per gram of adenoidal tissue did not differ significantly between the individual groups (tested by analysis of variance).

If the three bacterial species were combined and just considered as middle ear pathogens, the colonization rate for group 1 was 85%, for group 2, 92%, and for the control group, 66% (Table 3). By the χ^2 test there was no statistical difference among the individual groups; however, combining the children affected with present or previous ear disease revealed a significantly higher colonization rate for middle ear pathogens compared to normal children. Two or more pathogens were present at the same time in 33% to 58% of all adenoids.

Potential middle ear pathogens were only occasionally detected. The species recovered were mainly β -hemolytic group A streptococci, *S aureus*, and, rarely, *H influenzae* type B and streptococci groups C, G, or F. In an attempt to semiquantitatively determine any displacement of the normal flora, we graded

the colonization rate of α -hemolytic *Streptococcus* from mild to moderate to severe. There was no significant difference in children colonized with pathogenic bacteria.

The overall colonization rate for *M catarrhalis* was significantly higher during the winter months (by January) as compared to late fall (October). Whereas there was almost no variation regarding nontypeable *H influenzae*, *S pneumoniae* did show a clear tendency toward higher colonization of the adenoids during the winter months; however, the numbers did not reach statistical significance.

Bacterial adherence to the mucosa of the nasopharynx may involve specific receptor sites on the host cell surface. Our previous investigations using the chinchilla model had suggested that pneumococcal exoenzymes alter the cell surface carbohydrate structure and thereby expose part of the pneumococcal receptor structure.⁵ We therefore analyzed the adenoidal cell surface using five different lectins specific for terminal neuraminic acid (NeuAc; *Sambucus nigra* agglutinin, wheat germ agglutinin [WGA]), galactose (peanut agglutinin), and N-acetyl-glucosamine (GlcNAc; succinylated WGA, *Bandeiraea simplicifolia* lectin II) sugar residues. Whereas WGA binds both NeuAc and GlcNAc, succinylated WGA — due to its negative charge — does not bind NeuAc, though it retains its specificity toward GlcNAc.

The lectin staining revealed no significant differences between children suffering from recurrent OM

TABLE 5. ASSOCIATION OF SUCC WGA-POSITIVE LABELING AND COLONIZATION WITH *STREPTOCOCCUS PNEUMONIAE*

	Succ WGA- Positive	Succ WGA- Negative	χ^2 Test
<i>Streptococcus pneumoniae</i> -positive	85%	14%	NS
<i>Streptococcus pneumoniae</i> -negative	31%	68%	NS

Data are percentages of 52 immunohistochemically stained adenoids.
Succ WGA — succinylated wheat germ agglutinin, NS — not significant.

and children with no previous ear disease (Table 4). Colonization with *S pneumoniae* showed a clear tendency toward succinylated WGA-positive staining, indicating the partial removal of terminal sialic acid and the exposure of GlcNAc (see Figure). This observation, however, was not statistically significant (Table 5).

DISCUSSION

Bacterial adherence to mucosal surfaces is the first step in the colonization process and may lead to subsequent inflammation of the underlying tissue. The colonization of the nasopharynx starts soon after birth, with 44% of normal children becoming colonized with nontypeable *H influenzae* by the age of 2 years.⁶ Since a predominant strain is carried for only a few weeks, sequential colonizations with different strains occur frequently.

Whereas many children are asymptomatic carriers of possible middle ear pathogens, an increased nasopharyngeal colonization of pathogens has been found in children with AOM,⁷ in children with a previous history of suppurative and serous OM,⁸ and in children with persistent OME.⁹ A displacement of the normal flora (primarily of α -hemolytic *Streptococcus*) with potential middle ear pathogens has been observed in otitis-prone children as compared to non-otitis-prone children.¹⁰ We did not detect a significant alteration of the normal flora using a semiquantitative analysis; a conclusion, however, cannot be drawn, due to the lack of an absolute quantitative bacterial count for α -hemolytic *Streptococcus*.

Previous studies suggested that bacteria adhere mainly to the nonciliated cells of the nasopharynx and do not invade the tonsillar tissue.^{11,12} Our results indicate that children suffering from rOME or cOME demonstrated a significantly increased nasopharyngeal colonization rate of middle ear pathogens as compared to children with adenoidal hypertrophy only. The predominant pathogen was nontypeable *H influenzae*, followed by *S pneumoniae* and *M catarrhalis*. Nontypeable *H influenzae* was the predominant pathogen in children suffering from persistent

OME. *Streptococcus pneumoniae* showed the highest colonization rate in children with a previous history of rOME or rAOM; however, there were no statistical differences between the three groups. *Moraxella catarrhalis* revealed the highest colonization rate in children with a previous history of rOME or rAOM, with a significant overall increase of nasopharyngeal colonization during the winter months. The mean bacterial concentration was in the range of 10^5 cfu/g of adenoidal tissue. Statistical analysis revealed no significant differences between children with adenoidal hypertrophy only and children with concomitant ear disease. These results indicate that there is no nasopharyngeal overgrowth of pathogenic organisms in children with recurrent or persistent OM. The fact that many children were asymptomatic carriers of nontypeable *H influenzae* (57%), *S pneumoniae* (19%), and *M catarrhalis* (28%) with a similar quantitative culture rate of 10^5 cfu/g of adenoidal tissue further implies a multifactorial pathogenesis for OM.

Divergent data exist in the literature. This may be due to different sampling techniques¹³ (nasopharyngeal swabs versus quantitative bacteriology performed on adenoidal tissue, conventional culture techniques versus polymerase chain reaction) and different patient cohorts. Whereas prospective studies indicate a higher colonization rate of nontypeable *H influenzae* in otitis-prone children suffering from recurrent suppurative OM, the other two major pathogens (*S pneumoniae* and *M catarrhalis*) did not show a significantly increased colonization rate as compared to rates in control children.¹⁴⁻¹⁶ Other investigators noticed an age-related decrease of nasopharyngeal colonization in older children.^{11,17} Quantitative bacterial analysis of adenoidal tissue by Pillsbury et al⁸ revealed a significant relationship between infected adenoids showing a bacterial count greater than 10^5 cfu/g of tissue and a history of suppurative OM. Surprisingly, the predominant organisms were α - and β -hemolytic *Streptococcus*, *Neisseria* sp, and *S aureus*, whereas *S pneumoniae* and nontypeable *H influenzae* were rarely encountered. These data were not supported by the Swedish group,¹⁷ which found even higher concentrations of nontypeable *H influenzae* in the adenoidal hypertrophy group than in the OME group, and similar counts between the groups for *S pneumoniae* and *M catarrhalis*. Others depicted a clear tendency for greater numbers of colonies of nontypeable *H influenzae* and *S pneumoniae* in the otitis-prone groups; however, it did not reach statistical significance.^{10,18} It therefore appears that children at a young age who suffer from rAOM harbor more pathogenic bacteria in the nasopharynx than normal children or children with cOME. Adenoidal

infection remains difficult to verify. The present study could not validate a bacterial overload in adenoidal tissue of patients with recurrent or persistent ear disease.

The tissue tropism of many infectious agents can be explained by the recognition of specific receptor sites. Epidemiologic studies confirm the association of viral upper respiratory tract infections and bacterial OM. Viral and bacterial enzymes might modify and expose receptor structures on the epithelial cell surface, facilitating further adherence of pathogenic bacteria.⁵ In the present study, lectin staining of the adenoidal cell surface failed to demonstrate a significant difference between the three groups; however, colonization with *S pneumoniae* revealed exposure of terminal GlcNAc, which is part of the pneumococcal receptor structure on the cell surface,¹⁹ as has been observed in an animal model.⁵

Adenoidectomy has proved to have a beneficial effect on rAOM and on long-term resolution of cOME, and has reduced the need for reinfections of ventilation tubes.^{20,21} The removal of an infectious source in the nasopharynx is the pathophysiologic concept. Our study demonstrated an overall increased nasopharyngeal colonization rate of middle ear pathogens in children with present or previous recurrent ear disease as compared to children with adenoidal hypertrophy only. On the basis of these results, we support the concept of adenoidectomy in order to remove a bacterial focus. Bacteriologic data in the literature provide evidence that children at a young age are more frequently colonized. Further studies are therefore indicated to determine the effect of adenoidectomy in children younger than 4 years of age. Vaccination trials may also give further insight into the role of "adenoidal infection" in the pathogenesis of OM.

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DISTRIBUTION AND HERITABILITY OF RECURRENT EAR INFECTIONS

KARI J. KVAERNER, MD
JENNIFER R. HARRIS, PhD

KRISTIAN TAMBS, PhD
PER MAGNUS, MD

OSLO, NORWAY

The distribution of recurrent ear infections was obtained from a population-based sample of 2,750 pairs of Norwegian twins born between 1967 and 1974. The lifetime prevalence of self-reported recurrent ear infections was 8.9%, with a significant predominance of female cases. The mean age of onset was 4.2 years, with a gradual decrease in occurrence from 2 to 7 years of age. Among monozygotic pairs, the rate of tetrachoric correlation between co-twins was almost identical in males (0.73, SE 0.08) and females (0.74, SE 0.06), but among the dizygotic pairs the correlation was clearly higher in males (0.53, SE 0.12) than in females (0.20, SE 0.12). The value in the unlike-sexed dizygotic twins (0.25, SE 0.05) was intermediate to that of the like-sexed male and female dizygotic pairs. The relative contribution of genes and environment to variability in the predisposition to develop otitis media was estimated by means of structural equation modeling. Variation in liability to ear infections was mainly explained by additive genetic and dominance factors in females, for whom heritability was estimated at 74%. The remaining 26% of the variation in liability was explained by individual environmental factors. In males, 45% of the variation could be accounted for by genetic factors, 29% by common familial environment, and the remaining 26% by individual environmental effects.

KEY WORDS — acute otitis media, environment, genes, prevalence, twins.

INTRODUCTION

Otitis media is a common disease in children. Approximately 70% of all children have had at least one episode of acute otitis media (AOM) before their fifth birthday.^{1,2} Efforts have been made to identify otitis-prone children. Early age at onset of the first episode of AOM is considered an important risk factor, suggesting an underlying anatomic, physiologic, or immunologic dysfunction, or combinations of these, of the upper respiratory tract.^{1,2} Findings from several different types of studies implicate genetic factors in the causation of AOM. For instance, ethnic group differences have been reported, with relatively high incidences of AOM among American Indians, Alaskan and Canadian Eskimos, Australian Aborigines, and Africans.^{3,4} Family studies reveal that children whose siblings or parents have a history of recurrent acute otitis media (rAOM) are themselves at increased risk for developing rAOM.² Finally, results from a clinical study⁵ indicate a relationship between rAOM and the HLA-A locus. Specifically, the following antigen levels were compared between 45 children with rAOM and a healthy control group: 8 HLA-A, 15 HLA-B, 5 HLA-C, and 6 HLA-DR. The frequency of the HLA-A2 antigen was significantly higher among the group with rAOM (relative risk [RR] = 3.15), while the HLA-A3 antigen occurred at a significantly lower frequency.

Otitis media is an important public health problem

because of the morbidity, social costs, and consequences with which it is associated. Exploration of the genetic and environmental contributions to otitis media may be helpful for identifying children at risk and for planning intervention and treatment strategies. Although twin studies of AOM are not reported in the literature, the twin design offers a powerful approach to study genetic influences on AOM. The relative magnitude of genetic and environmental contributions to differences in the susceptibility to develop AOM can be estimated through an analysis of variation within and between twin pairs. In this study we use data from a population-based sample of twins to describe the distribution of recurrent episodes of AOM by age and sex; estimate the extent to which genes and environment contribute to variation in liability for recurrent ear infections and test for sex differences in these genetic and environmental effects; and investigate how parental information and co-twin contact may affect reporting and recall bias in these data.

MATERIALS AND METHODS

Sample. The sample was established from the Medical Birth Registry of Norway. In total, 5,078 twin pairs were born between 1967 and 1974. In 1992, questionnaires were sent to the 3,996 twin pairs who met the following criteria: both twins were alive and at least 18 years old, and current addresses were

From the Division of Epidemiology, National Institute of Public Health, Oslo, Norway. This research is supported, in part, by grants from the Norwegian Research Council (NFR).

CORRESPONDENCE — Kari J. Kvaerner, MD, National Institute of Public Health, Geitmyrsveien 75, N-0462 Oslo, Norway.

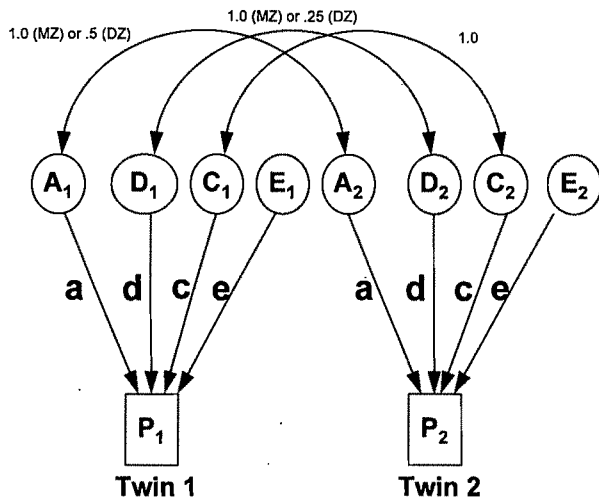


Fig 1. Path model depicting contribution of genetic and environmental factors to twin resemblance.

available from the National Census Registry. The individual response rate was 73% and included responses from 5,140 twins (2,570 intact pairs) plus 724 responses from individuals whose co-twin did not return the questionnaire. The respective pairwise and individual response rates were 60% and 69% among members of male-male pairs, 73% and 79% among members of female-female pairs, and 61% and 72% among members of unlike-sexed pairs.

Zygosity classification was based on answers to seven questionnaire items that have been shown to categorize correctly in more than 97% of cases in other Norwegian twin cohorts.⁶ The sample and zygosity classification procedure is more thoroughly described elsewhere.⁷ Among the 2,570 twin pairs with complete data, 944 were classified as monozygotic (MZ) and 1,626 as dizygotic (DZ).

Measurement. The questionnaire included the following three questions about ear infections: "Do you have, or have you had recurrent infections in the ear? If yes, do you remember the age of onset of the first infection? If the ear infections have stopped, at what age was the last episode?" Using the clinical criterion of 7 years or younger for age at onset, only those individuals reporting problems before the age of 7 were defined as cases, and the 120 persons who were older than 7 at their first reported age of onset were treated as noncases.

In order to conduct a validity check of the data, a measure of twin contact was constructed from six of the questionnaire items that asked about quality and degree of contact between co-twins. These questions inquired about frequency of personal contact, frequency of telephone contact, perceived closeness during life, years together in the same school class, age when they left home, and distance between cur-

rent residences. Pair means were used when both twins had complete data. If data from one twin were missing, data from the co-twin were used. The overall contact indicator was scored for each pair as the weighted sum of the scores on each contact item. The procedure for the creation of this indicator has been detailed elsewhere.⁸

Analyses. The distribution of ear infections according to age and sex was analyzed with data from complete pairs as well as from single responders. Co-twin similarity was measured with tetrachoric correlations estimated by the program Prelis.⁹ Computation of the tetrachoric correlations is based on the assumption of an underlying, normally distributed liability, and disease is manifest only when a certain liability threshold value is exceeded. The expected value for the tetrachoric correlation between two dichotomous variables is identical to the expectation for the Pearson correlation between the corresponding underlying normally distributed liabilities. The correlations are estimated separately for MZ and DZ pairs and then analyzed according to the model described below. The model descriptions, including the path model, specifications, and model input, have been detailed elsewhere,¹⁰ and will be briefly reviewed here.

A path model specifying how genes and environment contribute to twin resemblance is depicted in Fig 1. The phenotype (in our study, liability for ear infections) for each twin within a pair is specified as P1 and P2. Variation in the phenotype can be decomposed into unobserved genetic and environmental factors that are depicted in Fig 1 in circles, and subscripted with a 1 for the first twin and a 2 for the co-twin. Genetic effects are subdivided into two categories: *additive* (*a*) and *dominance* (*d*). Additive effects refer to intralocus gene action in which each allele in the pair is equally important for the expression of the phenotype. Dominance refers to intralocus interactions in which one allele exerts a greater influence in the expression of the phenotype. Environmental effects are further classified into those that are *common* (*c*) or *specific* (*e*) to the twins in a pair. Common environmental effects are those shared by both members of the pair, and include all sources of shared environment, from in utero effects to growing up in the same family. In contrast, specific environmental factors are all of the unique individual experiences that are not shared by co-twins, and contribute to within-pair differences.

The purpose of using twin data is to decompose phenotypic variation into portions due to genetic and environmental factors. These estimates are obtained by specifying a model that describes how the genetic

and environmental factors contribute to twin similarity, which is measured here by using the tetrachoric correlations. The double-headed arrows in Fig 1 indicate that genetic and environmental effects for the phenotype in twin 1 are correlated with the same effects in twin 2. The intrapair correlation for genetic factors is based on the degree of biologic relatedness. Monozygotic twins share all of their genes and, thereby, both additive and dominance effects are perfectly correlated in MZ twins and contribute only to within-pair similarity. However, DZ twins share approximately half of their segregating genes, corresponding to a correlation at 0.5 for additive genetic effects and 0.25 for the effects of dominance. Thus, among the DZ pairs, genetic factors contribute to intrapair similarities and differences. In addition to shared genetic effects, twin resemblance can result from shared environmental effects. By definition, common environments are perfectly correlated in all twin pairs regardless of zygosity. Finally, specific environmental factors contribute only to differences within pairs.

The analysis procedure fits structural equation models based on path analysis to the observed correlations. Estimates of the genetic and environmental contributions to the variation in liability are represented by their respective path coefficients connecting the factors to the phenotype in Fig 1. The path coefficients, designated a , d , c , and e , are standardized partial regression coefficients. The proportion of total phenotypic variance attributable to each factor is obtained by squaring the value of the standardized path coefficient. Heritability (the proportion of variance in liability of recurrent ear infections explained by all sources of genetic differences) can be estimated as $h^2 = a^2 + d^2$, and the total variance due to environmental effects is $c^2 + e^2$.

This basic model implies that the proportion of variation explained by each of the genetic and environmental factors does not differ between males and females. Thus, there is only one estimate for each of the path coefficients a , c , d , and e . However, this model can be expanded to test whether the genetic and environmental variance structure differs for males and females. In this application, the tetrachoric correlations are calculated separately for the five groups defined by the sex of the pair (male-male, female-female, and male-female) and zygosity, and the magnitude of the genetic and environmental parameters are allowed to vary between the sexes. Now the model postulates that genetic factors affecting ear infections in males (AM) may not be identical with those in females (AF), and separate parameter estimates are derived for males and females. The corre-

lation coefficient, ra , represents the degree to which the same genes are contributing to variation in ear infections for males and females.

Models depicted by the path diagram in Fig 1, and expanded to estimate male and female parameters for genetic and environmental effects, were fitted to the observed twin correlations with a program based on the SAS NLIN computer procedure (SAS/STAT, 1988), originally prepared by Eaves.¹¹ More specifically, the model is composed of a set of linear equations that represent twin resemblance, measured by the correlation r , as a function of shared genetic and environmental factors.

$$r_{MZM} = aM^2 + dM^2 + cM^2$$

$$r_{MZF} = aF^2 + dF^2 + cF^2$$

$$r_{DZM} = 1/2 aM^2 + 1/4 dM^2 + cM^2$$

$$r_{DZF} = 1/2 aF^2 + 1/4 dF^2 + cF^2$$

$$r_{DZU} = 1/2 aMaFra + 1/4 dMdF + cMcF$$

The parameter e is not a free parameter; however, by definition $aM^2 + dM^2 + cM^2 + eM^2 = aF^2 + dF^2 + cF^2 + eF^2 = 1$, and e can be calculated as a function of the other parameter estimates. Identification of the model requires that the number of free parameters does not exceed the number of observed statistics, so that there is sufficient information to estimate each parameter uniquely. Therefore, not all parameters could be estimated simultaneously, and several models are tested that alternatively exclude parameters. Full models with a maximal number of parameters were tested sequentially. Only one full model will usually fit the data perfectly, and this model was compared to nested models in which additional parameters were fixed at zero. At first dM and dF were fixed at zero, next, cM and cF , then dM and cF , and finally cM and dF were fixed at zero. To test whether a particular parameter (genetic or environmental effect) is significant, the fits of two nested models are compared by the χ^2 difference test with one degree of freedom. The parameter is significant if the fit of the model that fixes the parameter to zero is significantly worse than that which estimates the parameter. A thorough description of the methods used in this study is given elsewhere.¹⁰

With data on disease during childhood reported retrospectively, recall bias may affect the results. Information provided by the parents could affect the reports, most likely by identifying cases that would otherwise have been ignored, and thus increasing the validity of the information. Exchange of information between co-twins (reporting cooperation) may also have improved the validity, but could simultaneously

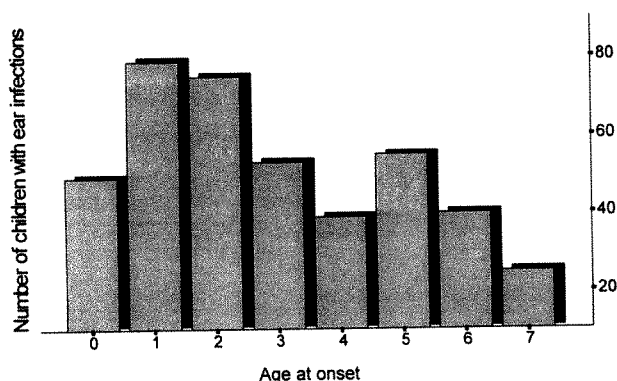


Fig 2. Distribution of recurrent ear infections before age of 7.

inflate co-twin resemblance, because some participants could have erroneously assumed that their co-twin's answer was valid for both twins. Such effects were explored by comparing *prevalence* in: 1) twins still living in their family home with twins who had moved, and 2) twins with high versus low personal contact as estimated by an overall contact indicator described earlier. If access to parental information increases the validity of the data, then the reported prevalence is expected to be higher in twins who live at home than in twins with less access to parental information, because lack of information is likely to cause more false negatives than false positives. Regarding reporting cooperation in co-twins, it is difficult to predict in which direction, if any, such exchange of information would affect reported prevalence. Nevertheless, a significant difference in prevalence between twins in the higher- versus the lower-contact group would signal some type of reporting bias.

The twin correlations were recomputed in subgroups in order to explore whether reporting cooperation or parental information may have affected the results from the *genetic analyses*. First, to test for reporting cooperation, correlations were computed separately among pairs reporting higher contact and among those reporting lower contact. Each zygosity group (MZ, like-sexed DZ, and unlike-sexed DZ) was divided into two (almost) equal-size groups according to degree of reported contact. Models were fitted separately to the correlations in the subgroups with lower-contact pairs and then in higher-contact pairs. Next, the model was fitted to the two sets of correlations simultaneously. The difference between the sum of the χ^2 value from the analyses of the separate data and the χ^2 value from the analysis of the combined data provides a test of the heterogeneity between the separate sets of correlations. If the two correlation sets differ significantly, the differences between the parameter estimates based on the lower-contact groups and the estimates based on the higher-

contact groups will give an indication of the *extent* to which reporting cooperation may have biased the results.

As with close contact, living together in the childhood home may increase the chances of reporting cooperation. Also, greater access to parental information could inflate the co-twin correlations in twins still living in their childhood home if parental misinformation biased both twins' reports in the same direction. Some parents may tend to remember that both twins were sick, or that both were well, when only one of the twins was actually affected. But greater access to parental information may also have improved the validity of the reports, and thereby increased the correlations for twins living with their parents. In this case, higher correlations result from reduced attenuation of measurement error. Thus, higher correlations among pairs living with their parents than among pairs living away from home could be caused by bias effects or by greater validity in reports, whereas correlations of similar magnitude among pairs living together and pairs living away from home would indicate that neither improved validity nor bias effects specific to twins living together are important.

There is a considerable overlap between co-twin contact score and the variable "living together/not living together in childhood home" (point-biserial correlation .52). Because the overlap is far from perfect, we found it worthwhile to examine possible effects of each measure separately. It would be helpful to apply the same procedure used for testing the effects of higher versus lower contact with the pairs categorized as living together versus living apart. However, due to the sample restrictions in the low number of pairs living together, particularly female DZ twins, those data were not suited for model testing.

RESULTS

Of the respondents, 8.9% (519) reported having, or having had, recurrent ear infections that started before the age of 7. The distribution for age of onset for ear infections is presented in Fig 2; in 50% of the cases the first infection appeared during the first 3 years of life, and the mean age of onset was 2.7 years. There was a significant predominance among females in the prevalence of ear infections, with 10.6% among females compared to 6.9% among males ($\chi^2_1 = 25.2$, $p < .001$). The reported prevalence, shown in Table 1, was significantly higher among pair than single responders ($\chi^2_1 = 14.2$, $p < .001$). Neither the effects of living together in the family home nor the effects of contact (contact scores in the upper 50% versus the lower 50%) were significant ($\chi^2_1 < 3.84$,

TABLE 1. PREVALENCE OF RECURRENT EAR INFECTIONS PRIOR TO AGE SEVEN

Sex and Zygosity	Prevalence Among Pair Responders			Prevalence Among Single Responders			Prevalence Among All Responders		
	<i>n</i>	%	<i>N</i>	<i>n</i>	%	<i>N</i>	<i>n</i>	%	<i>N</i>
Males	177	7.4	2,402	13	3.6	363	190	6.9*	2,765
Females	305	11.1	2,738	24	6.6	361	329	10.6	3,099
All MZs	190	10.1	1,888	2	2.3	88	192	9.7	1,976
All like-sexed DZs	153	9.2	1,660	14	4.1	345	167	8.3	2,005
All unlike-sexed DZs	139	8.7	1,592	21	7.2	291	160	8.5	1,883
Total	482	9.4*	5,140	37	5.1	724	519	8.9	5,864

MZs — monozygotic pairs of co-twins, DZs — dizygotic pairs of co-twins.

* $p < .001$.

$p > .05$).

The median reported duration of the disease was 6.0 years, and durations for the upper and lower quartiles were 2 and 8 years, respectively. Sex differences in duration of ear infections were not significant.

Among the MZ twins, 52 pairs were concordant and 113 pairs were discordant for ear infections before the age of 7, and the pairwise concordance rate was 0.34. Among the DZ twins there were 40 concordant pairs and 277 discordant pairs, yielding a concordance rate of 0.13. Tetrachoric correlations, standard errors of the correlations, and numbers of pairs are listed in Table 2. The correlations were almost identical among MZ male and female pairs, but were significantly higher in the DZ males than females ($z > 1.96$, $p < .05$) and were intermediate to the male-female value in unlike-sexed DZ twins.

A series of structural equation models was fitted to the twin correlations. In a model specifying sex-specific genetic additive (aM and aF) and dominance (dM and dF) effects and individual environmental effects (eM and eF), the effect of genetic dominance in males (dM) was estimated to be zero. In a model with sex-specific genetic additive effects and common (cM and cF) and individual (eM and eF) environmental effects, the common environmental effects in females (cF) were estimated to be zero. Therefore, further model

TABLE 2. CO-TWIN CORRELATIONS, STANDARD ERRORS, AND NUMBERS OF PAIRS BY SEX AND ZYGOSITY

Tetrachoric Correlations by Sex and Zygosity	<i>r</i>	<i>SE</i>	<i>N of Pairs</i>
Male MZs	0.737	0.08	416
Female MZs	0.740	0.06	528
All MZs	0.743	0.05	944
Male DZs	0.528	0.12	387
Female DZs	0.195	0.12	443
All like-sexed DZs	0.337	0.09	830
All unlike-sexed DZs	0.249	0.08	796

testing started with a combined full, or saturated, model specifying dominance in females (dF) and common environment in males (cM). Standardized estimates of genetic and environmental variance components (squared parameter estimates) and the model-fit statistics for this model (model 1) and subsequent models are presented in Table 3. The Akaike Information Criterion, $AIC = \chi^2 - 2df$,¹² provides a statistic for comparative fits across models with various degrees of freedom. Models 1 through 8 test successively for the importance of genetic dominance in females (dF); common environment in males (cM); both dF , and cM , additive genetic effects in females (aF); and sex differences in genetic ($aM = aF$) and nonshared environmental effects ($eM = eF$). A fifth parameter, ra , is the correlation between additive genetic effects in males and females. If ra is estimated to be less than unity, then there is evidence that different sets of genes contribute to variation in AOM in males and females. In the first completely saturated model, ra was estimated to be 1.0; thus, this correlation was fixed at unity in all subsequent models containing aM and aF . A test of genetic dominance in females, dF , was conducted by dropping dF from model 3 and comparing the fit to that of model 2. Results indicated that dF was not significant ($\chi^2_1 = 1.79$, $p = .181$, comparing model 3 with model 2). Also, the effect of common environment in males, cM , could be fixed at zero without a clearly significant loss of fit ($\chi^2_1 = 2.91$, $p = .180$, model 4 minus model 2). Next, we tested a model in which both dF and cM were fixed at zero. Results indicated that both parameters could not simultaneously be left out without significant loss of fit ($\chi^2_2 = 7.01$, $p = .030$, model 5 minus model 2). Additive genetic effects were significant for both sexes, and could not be dropped from the model for males ($\chi^2_1 = 14.50$, $p = .000$, model 6 minus model 2) or females ($\chi^2_1 = 6.12$, $p = .014$, model 7 minus model 2).

Models 1 through 7 allow the magnitude of genetic and environmental effects to vary in males and females. Next, a series of models was analyzed that

TABLE 3. GENETIC AND ENVIRONMENTAL VARIANCE ESTIMATES AND MODEL-FITTING STATISTICS

Model	a ² M	a ² F	raMF	c ² M	d ² F	e ² M	e ² F	χ^2	df	p	AIC
1	0.452	0.364	1	0.287	0.374	0.261	0.262	0.81	0		
2	0.452	0.364	1*	0.287	0.374	0.261	0.262	0.81	1	.368	-1.19
3	0.395	0.737	1*	0.341		0.264	0.263	2.60	2	.273	-1.40
4	0.744	0.274	1*		0.466	0.256	0.260	3.72	2	.156	0.28
5	0.741	0.735	1*			0.259	0.265	7.82	3	.050	1.82
6		0.040		0.698	0.699	0.302	0.261	15.31	2	.000	13.31
7	0.418			0.319	0.740	0.263	0.260	6.93	2	.313	4.93
8	0.736	0.736	1*			0.264	0.264	7.86	4	.189	-0.14

*Correlation between male and female genetic effects fixed at unity.

placed equality constraints on the parameters for males and females. In a model with a , d , and e and all parameters constrained to be equal in males and females, the dominance effect could be dropped with a negligible increase in χ^2 ($\chi^2_3 = 7.46$ versus $\chi^2_4 = 7.86$). In a model with a , c , and e , common environment could be dropped without any increase in χ^2 . Because the model fit was almost unaffected by excluding dominance and common environment, only the results from the model equating aM with aF , and eM with eF (model 8) are shown.

As revealed in Table 3, models 2 through 4 are approximately equal and fit the data well. Because we could not drop both CM and DF from the model simultaneously, we decided to keep the model with both parameters, which is model 2. The results from model 2 indicate that variation in liability of ear infections is explained by additive genetic effects, common familial environment in males, dominance effects in females, and effects of individual environment. In males, genetic effects explain 45% of the variation, common familial effects account for 29%, and individual environmental factors explain the remaining 26%. For females, approximately 37% of the variation in ear infections is attributable to additive genetic effects, 37% is attributable to dominance effects, and the remaining variation is accounted for by nonshared environment.

The effect of personal contact between the twins was examined by comparing twins in higher- and

lower-contact groups. The correlations tend to be greater in twins with more co-twin contact than in twins with less co-twin contact. However, these differences were not significant in any of the zygosity or sex subgroups. Heterogeneity of the correlations in the high- and low-contact groups was tested by computing the difference between the χ^2 value from the analysis of the combined data and the summed χ^2 value from the analyses of the separate data. Table 4 shows the results from fitting the basic model to each of the data sets separately (higher and lower personal contact) and then to both sets simultaneously. The parameter for the genetic correlation could be fixed at 1.0 almost without any χ^2 increase for the two separate as well as the combined data sets. The increase in χ^2 from the analysis of the combined data compared to the sum of χ^2 from the separate fits of each data set is significant ($\chi^2_4 = 13.39$, $p = .010$), implying a systematic difference in twin resemblance in twins with closer contact versus those with less close contact. Specifying an additional parameter, c' , for effects of environmental factors shared only by co-twins in the close-contact group restored the model fit. The new parameter accounts for approximately 12.3% of the variation in the higher-contact group.

Finally, the effect of living together in the childhood home was explored by comparing twins living at home with twins living away from their parents. The twin correlations in the two groups are shown in Table 5 by sex and zygosity. The twin correlations tend to be greater in pairs still living at home. With the

TABLE 4. MODEL-FITTING RESULTS TESTING FOR HOMOGENEITY OF GENETIC AND ENVIRONMENTAL EFFECTS IN PAIRS WITH LOW VERSUS HIGH CONTACT

	a ² M	a ² F	c ² M	d ² F	e ² M	e ² F	c' ²	χ^2	df	p	AIC
Low personal contact	0.471	0.470	0.203	0.170	0.326	0.360		3.07	1	.080	1.07
High personal contact	0.473	0.289	0.313	0.540	0.214	0.171		0.24	1	.624	-1.76
Both data sets combined	0.500	0.387	0.261	0.429	0.239	0.181		16.70	6	.010	4.70
Added contact parameter	0.511	0.199	0.155	0.502	0.211	0.176	0.123	5.60	5	.347	-4.40

Note: raMF fixed at 1.0 for all models.

TABLE 5. CO-TWIN CORRELATIONS BY RESIDENTIAL STATUS

	Residence in Parental Home			One or Both Twins Moved From Home		
	<i>r</i>	<i>SE</i>	<i>n</i> (pairs)	<i>r</i>	<i>SE</i>	<i>n</i> (pairs)
Male MZs	0.772	0.10	164	0.689	0.12	252
Female MZs	0.864	0.08	120	0.701	0.07	408
All MZs	0.813	0.07	284	0.708	0.06	660
Male DZs	0.456	0.20	140	0.587	0.14	247
Female DZs	0.637	0.19	74	0.069	0.14	369
All like-sexed DZs	0.537	0.14	214	0.262	0.11	616
All unlike-sexed DZs	0.138	0.15	394	0.333	0.13	402

asymptotic standard errors provided by the computer program used as indicators of the variability of the correlation estimates, the difference was significant for DZ females ($z = 2.40$, $p = .016$). The estimation of tetrachoric correlations and their standard errors can be unreliable in samples smaller than 200 cases, especially when the distribution is markedly skewed, such as for ear infections.^{11,12} In our sample, the number of female DZ pairs living together is only 74; thus, the result of the significance test must be interpreted with caution. Ideally, one would test for heterogeneity of the twin correlations according to whether the pair lives at home or not. However, the small numbers of pairs in some subgroups living in the family home, particularly female DZ pairs, prevent such tests in these data.

DISCUSSION

Genetic and environmental sources of variance for recurrent ear infections were explored in a population-based sample of 18- to 25-year-old Norwegian twins. To our knowledge, this is the first twin study of heritability for recurrent ear infections.

Prevalence. The prevalence of self-reported recurrent ear infections with onset prior to age 7 was 8.9%, with a significant predominance in females (10.6%) compared to males (6.9%). The mean age of onset was 2.7 years, with a gradual decrease in prevalence from 2 to 7 years of age.

Genetic and Environmental Influences. Previous evidence for genetic predisposition for ear infections comes from familial studies reporting that children with siblings or one parent with a history of rAOM are at increased risk of contracting rAOM.^{2,4} A clinical study reports a relationship between rAOM and the HLA-A locus,⁵ suggesting the involvement of genetic factors. A genetic influence may exert its effect through skull anatomic differences, changes in the

eustachian tube function, or an immunologic predisposition. Among the proposed etiologic factors in otitis media is a variation in skull morphology leading to a change in the normal nasopharyngeal proportions. Lateral cephalometric analysis of children with secretory otitis media (SOM) reveals a significant reduction in certain skeletal and soft tissue dimensions in the nasopharynx, and a reduced or delayed rate of localized skull growth.¹³ It has been postulated that variations of resorption and deposition in each skull bone in amount, rate, and timing between individuals are mainly genetically determined and to a lesser degree affected by functional tissue relationships.¹⁴ Genetic effects may also contribute substantially to variation in the dimensions in the nasopharynx, and may therefore explain why the estimate of heritability is large in the present study.

Genetic effects may also contribute to immunologic predisposition. Apparently, specific secretory antibodies are produced in the middle ear mucosa during AOM. The presence of such antibodies is associated with termination of the disease. These antibodies appear during a later phase of the disease in younger children than they do in older children.¹⁵ However, when the antibody response of otitis-prone children was compared to that of a healthy control group, the former had lower levels of immunoglobulin G2 at both 12 and 32 months of age, suggesting the importance of this immunoglobulin subclass in the duration and recurrence of otitis media in children.¹⁶

Several risk factors for otitis media have been postulated. Attending day-care nurseries and having siblings seem to be important risk factors.^{17,18} Thus, if environmental risk factors are considered to be important, this should be reflected in the common familial environment, c , as the co-twins are expected to have mainly the same environmental exposure in childhood. Our results suggest that an effect of common familial environment is present in males, but the same effect could not be shown in females. Males may be more vulnerable to, or exposed more often to, environmental risk factors than females. For instance, males engage in more rough play behavior, and may spend more time outdoors under adverse weather conditions. However, further elucidation of these explanations awaits studies of differential environmental exposure among boys and girls. Nonshared environmental factors certainly affect the development of otitis media. Approximately one quarter of the variation in liability appears to be explained by such factors. An important direction for further research is to identify specific sources of environmental variance in the nonshared domain for both sexes, and in the shared domain for males.

Other Considerations. The two important effects of recall and selection bias may be influencing our estimates of prevalence and genetic and environmental parameters. The significant predominance of ear infections in females stands in contrast to other clinical studies that show a slightly higher risk of developing AOM in males.^{1,2} This discrepancy may be due, in part, to underreporting in males and a more conscientious reporting tendency in females. Furthermore, there may be a selection bias toward more severe disease in clinical studies because a threshold of symptom severity has to be reached before a doctor is consulted. Such threshold values could vary somewhat between males and females, if, for instance, acceptance of distress is slightly higher in females than in males. If so, the population-based sample may give a more correct estimate of the prevalence of the disease than clinical data, and this could explain the higher prevalence of ear infections in females than in males in our sample.^{1,2}

The questions about ear infections are retrospective, and the reliability of the data may be affected by recall bias in two ways. First, do the respondents correctly recall whether they have had ear infections? And second, what does the questionnaire-based term "recurrent ear infections" mean to each responder? Respondents who report a positive history of ear infections could have had either AOM or SOM. It is less likely that they experienced otitis externa, because it is rare in children. With great probability, the majority of the cases are AOM, as SOM is not associated with pain, nor was it often treated with operative procedures when this population sample was younger than age 7. Furthermore, the age distribution suggests that a large majority of cases were AOM. In the present study, 82% had otitis media before the age of 7. Figures for AOM before 7 were 93% in a Boston study² and 70% in a Swedish study.¹ Additionally, our data show a reduction in the occurrence of otitis media from 2 to 7 years, and a similar pattern of decline in AOM was found by Klein² in Boston.

The prevalence of ear infections is significantly lower among the single compared to the pair responders, suggesting a systematic bias due to selection or misreporting. However, it is unlikely that selection toward ear infections could be strong on a three-page questionnaire that includes only three items about ear infections. Rather, the proportion of pairs concordant versus discordant for participation or nonparticipation suggests strong co-twin similarity in willingness to participate, and the lower frequency of reported cases in single responders may reflect a lack of motivation. Confirming illness that occurred early in life may

require some effort, such as contacting the parents. Most responders did not live with their parents when the questionnaires were completed, and a history of ear infection could simply be ignored by some weakly motivated responders. The tendency toward a greater prevalence of ear infections among twins who still were living at home supports this explanation. If so, the prevalence of 8.9% representing complete pair responders is more likely to be a slight underestimation than an overestimation.

Differing interpretations of the questions about ear infections, coupled with recall problems, may influence self-reports. In the case of measurement error, if the sources are random (ie, not correlated within the twin pair), then measurement error will inflate the estimate of nonshared environment, e , and correspondingly deflate the estimate of heritability. Consequently, the "true" heritability in this study will have been slightly underestimated.

Higher correlations in twins with closer contact compared to those with less-close contact, and in twins living with parents versus those who are not, could reflect a real effect of contact. If closer adult contact is related to closer childhood contact, then it is possible that co-twins with more contact also shared environmental influences to a greater degree than twins with less contact. However, in these data it is more likely that the elevated correlations among the twins in the high-contact group reflect reporting cooperation or other exchange of information. Similarly, higher correlations among twins who live with their parents than among those who live away from home may, in part, be attributed to reporting cooperation as well as high access to parental information. Both contact and living together could bias the results in a systematic manner. Whereas absence of parental information may artificially lower the correlations among twins living *apart* from their parents, parental misinformation affecting both twins in the same direction and reporting cooperation have probably *inflated* the correlations in twins living *with* their parents. The net effect may be that these influences counteract one another in their effect on the parameter estimates. In these data only a small percentage of the pairs still lived with their parents. The difference between correlations in the low- and high-contact groups suggests that at least some of the closest pairs may have cooperated during the completion of the questionnaires. Nonetheless, the degree of contact did not appreciably affect the parameter estimates. In sum, despite problems inherent in health data collected retrospectively, we did not find evidence of major bias effects.

Even though systematic bias effects may not have

been important, some of the estimates may have been subject to considerable fluctuation. Estimates of dichotomous and skewedly distributed variables such as ear infection require much larger samples than continuously distributed traits to acquire the same level of precision. With MZ and DZ twin data, the estimates of nonshared environment are fairly precise, those for total genetic effects are a little less precise, and estimates of genetic dominance and common environment are much less precise. This is apparent from inspection of the estimates for male common environment (model 3, Table 3) and female genetic dominance (model 4, Table 3). Both exceed one third of the total variance; nonetheless, tests of the effects of male common environment are not clearly significant, and tests of female genetic dominance do not even approach significance. The estimate of dominance among the females is based mainly on the difference between the correlations for MZ and DZ females and unlike-sexed DZ pairs. A DZ correlation of less than half the MZ correlation indicates genetic dominance. The correlation for unlike-sexed pairs is not too far from half the MZ correlation, and despite the low correlation among

the DZ females, the upper limit of the confidence interval for this correlation [$\approx r_{DZF} + (2 \times \text{the standard error}) = .44$] is well above half the MZ correlation. In general, rather substantial effects of both genetic dominance and common environment may remain undetected in studies like ours. The trends for genetic dominance among females and effects of common environment among males need replication from other studies.

In conclusion, the prevalence of self-reported recurrent ear infections with onset before 7 years was 8.9%, with a significant predominance in females (10.6%) compared to males (6.9%). Aside from possible bias effects and fluctuations, the results suggest that variation in liability for ear infections is explained mainly by additive genetic and dominance factors in females, for whom heritability is estimated at 74%. Individual environmental factors appear to account for the remaining variation among females. In males, the variation attributable to genetic factors was estimated at 45%, variance due to common familial environment at 29%, and variance explained by individual environmental effects at 26%.

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ROLE OF TUMOR NECROSIS FACTOR AND INTERLEUKIN-1 IN ENDOTOXIN-INDUCED MIDDLE EAR EFFUSIONS

STEVEN S. BALL, MD

JIRI PRAZMA, MD, PhD

RUDOLPH J. TRIANA, MD

C. G. DEAN DAIS, MD

HAROLD C. PILLSBURY, MD

CHAPEL HILL, NORTH CAROLINA

In a rat model, we investigated the role of tumor necrosis factor (TNF) and interleukin-1 (IL-1) in endotoxin-induced middle ear effusions (MEEs). After the eustachian tube was obstructed, the middle ear was transtympanically injected with 35 μ L of either 1) 1 mg/mL lipopolysaccharide (LPS); 2) LPS and 100 μ g TNF binding protein (TNFbp); 3) LPS and 1 μ g IL-1 receptor antagonist (IL-1ra); or 4) LPS, TNFbp, and IL-1ra. Every 2 hours, the fluid within the middle ear was collected, and the quantity of albumin in the fluid, an index of vascular leakage, was determined by enzyme-linked immunosorbent assay. After 6 hours, the middle ear was fixed for histologic analysis. The TNFbp significantly attenuated vascular extravasation into the middle ear. The IL-1ra did not significantly alter effusion development. These results indicate that TNF, but not IL-1, is a mediator of LPS-induced MEE. Therefore, TNFbp may represent a novel approach to the treatment of otitis media with effusion.

KEY WORDS — effusion, interleukin-1, lipopolysaccharide, middle ear, neutrophil, tumor necrosis factor.

INTRODUCTION

Otitis media with effusion (OME), the most common inflammatory disease of children after the cold, continues to cause significant sequelae despite aggressive medical and surgical treatment. It remains the most common cause of acquired hearing loss as well as impaired speech and language development in children.¹

The cause of OME is extremely complex; although OME was originally attributed to eustachian tube dysfunction, many other factors are now thought to contribute to OME.² For instance, some cases of OME are believed to be caused by bacterial remnants that are not cleared from the middle ear after an attack of acute otitis media has resolved.³

Bacterial endotoxin, a collection of gram-negative bacterial cell wall components with potent inflammatory properties, is highly suspect as a factor in the pathogenesis of OME. Lipopolysaccharide (LPS), the primary component of endotoxin, has repeatedly been isolated from the middle ear effusions (MEEs) of patients with OME⁴⁻⁶ and used to induce MEEs in animal models.^{3,7-11} The LPS stimulates these effusions by generating a localized inflammatory response within the middle ear; such a response in-

creases middle ear vascular permeability.^{3,7,12,13}

Lipopolysaccharide-induced inflammation is regulated by a complex network of inflammatory mediators, including cytokines.^{14,15} The most extensively studied cytokines are interleukin-1 (IL-1) and tumor necrosis factor (TNF). Both IL-1 and TNF have been isolated from human MEEs,¹⁶⁻¹⁸ and recombinant TNF has been used to induce MEEs in guinea pigs.¹⁹ However, neither IL-1 nor TNF has been shown to actively mediate the development of LPS-induced MEEs. The purpose of our study was to evaluate the role of IL-1 and TNF in LPS-induced MEEs with novel human cytokine antagonists; in a rat model, we attempted to suppress LP-induced MEEs with IL-1 receptor antagonist (IL-1ra) and TNF binding protein (TNFbp). These antagonists have recently been investigated extensively because of their potential for modulating many pathologic inflammatory disorders, including sepsis, arthritis, and meningitis.²⁰

MATERIALS AND METHODS

Thirty Sprague-Dawley rats, weighing between 250 and 275 g, were randomly divided into one control group and four experimental groups. In the control group, each rat's right middle ear was exposed only to Krebs-Ringer (KR) solution. In all of the

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CORRESPONDENCE — Jiri Prazma, MD, PhD, Division of Otolaryngology—Head and Neck Surgery, Dept of Surgery, University of North Carolina School of Medicine, 610 Burnett-Womack Building, CB# 7070, Chapel Hill, NC 27599-7070.

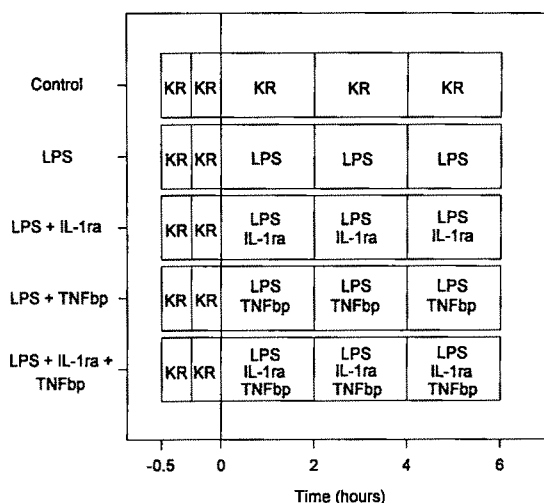


Fig 1. Protocol for middle ear effusion induction. After flushing each middle ear twice with Krebs-Ringer solution, middle ear was exposed to 35 μ L of solution designated in Figure. Every 2 hours, fluid within middle ear was collected, and middle ear was refilled with 35 μ L of designated solution. KR — 294 mOsm Krebs-Ringer solution, devoid of glucose; LPS — 1 mg/mL lipopolysaccharide in KR; IL-1ra — 100 μ g interleukin-1 receptor antagonist in KR; TNFbp — 1 μ g tumor necrosis factor binding protein in KR.

experimental groups, the middle ear was inoculated with LPS to induce an MEE. In three of these groups, the effusion was altered by IL-1ra or TNFbp or a combination of the two.

Materials. The LPS (*Pseudomonas aeruginosa* serotype 10 [Habs] L-9143) was purchased as a lyophilized powder from Sigma Chemical Co, St Louis, Mo. It was dissolved in a modified KR solution, devoid of glucose: 140 mmol/L sodium (Na^+), 120 mmol/L chloride (Cl^-), 5.2 mmol/L potassium (K^+), 25 mmol/L bicarbonate (HCO_3^-), 1.1 mmol/L calcium (Ca^{2+}), 1.2 mmol/L magnesium (Mg^{2+}), and 0.4 mmol/L phosphate (HPO_4^{2-}) in distilled water. A concentration of 1 mg/mL of LPS was used throughout this study. The IL-1ra and TNFbp were a gift from Synergen, Boulder, Colo. The IL-1ra was supplied in a 100 mg/mL solution of 10 mmol/L citrate buffer, 140 mmol/L sodium chloride, 0.5 mmol/L ethylenediaminetetraacetic acid (pH 6.5), and 0.1% Tween, and the TNFbp was supplied in an 8.8-mg/mL solution of the same citrate buffer. Both the IL-1ra and TNFbp were stored in solution at -20°C between experiments. Prior to each experiment, the IL-1ra and TNFbp were diluted in the modified KR solution noted above. Inoculates of 100 μ g IL-1ra and 1 μ g TNFbp in the modified KR solution were used throughout this investigation.

Surgical Preparation. Each rat was anesthetized with 0.1 mL/100 g of equal parts of 20 mg/mL xylazine and 100 mg/mL ketamine hydrochloride. The

right tympanic membrane of the rat was inspected to rule out the presence of a preexisting effusion. The rat was placed in the supine position. A ventral midline incision was made. A tracheostomy tube was inserted into the trachea. The left external jugular vein was catheterized and infused with 80 μ L/min/kg of 300 mOsm KR solution (140 mmol/L Na^+ , 120 mmol/L Cl^- , 5.2 mmol/L K^+ , 25 mmol/L HCO_3^- , 1.1 mmol/L Ca^{2+} , 1.2 mmol/L Mg^{2+} , 0.4 mmol/L HPO_4^{2-} , and 5.6 mmol/L glucose in distilled water) to maintain renal perfusion and systemic blood pressure throughout the experiment. Body temperature was maintained at 37°C by a thermistor-controlled heating pad.

Eustachian Tube Obstruction and Middle Ear Effusion Induction. The method we used to obstruct the right eustachian tube of each rat and to perforate the right tympanic membrane of each rat in preparation for effusion induction has been described.²¹ In essence, the eustachian tube was obstructed by drilling a 0.8-mm-diameter hole in the rostromedial process of the tympanic bulla, through which the eustachian tube courses, and packing the hole with dental wax. Subsequently, two perforations were made in the tympanic membrane, one in the posteroinferior quadrant and the other in the anterosuperior quadrant; the posteroinferior perforation was used to introduce fluid into the middle ear and recover it, and the anterosuperior perforation, to equilibrate middle ear pressure during fluid introduction and recovery.

Our protocol for MEE induction is diagrammed in Fig 1. For all five groups of rats, immediately after the tympanic membrane was perforated, the middle ear was flushed twice with 35 μ L of 300 mOsm KR solution to achieve a homeostatic middle ear environment.

Immediately after flushing the middle ear, the ear was transtympanically injected with 35 μ L of one of five solutions: 1) KR solution alone, the control solution ($n = 3$); 2) LPS ($n = 7$); 3) LPS and IL-1ra ($n = 6$); 4) LPS and TNFbp ($n = 7$); or 5) LPS, IL-1ra, and TNFbp ($n = 6$). The concentrations used were those cited in Materials. Over the next 6 hours, the fluid within the middle ear was collected every 2 hours and saved for analysis. Immediately after each collection, the middle ear was refilled with 35 μ L of solution identical to that previously injected into the ear.

Middle Ear Fluid Analysis. The volume of middle ear fluid collected at the end of each interval was measured with calibrated micropipets. The quantity of albumin in the fluid was measured with an enzyme-linked immunosorbent assay (ELISA).²¹ For

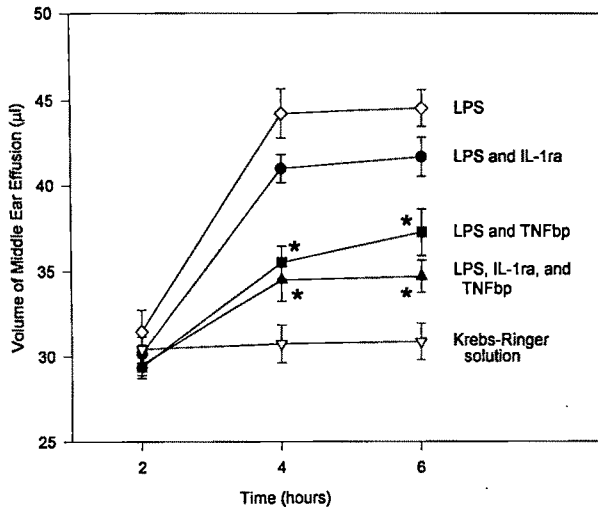


Fig 2. Mean volume of fluid collected from middle ears every 2 hours over 6-hour period. Middle ears were exposed to either KR; LPS; LPS and IL-1ra; LPS and TNFbp; or LPS, IL-1ra, and TNFbp. Asterisk designates mean volumes that were significantly different from corresponding mean volumes of group whose ears were exposed to LPS alone. Error bars represent standard error of mean (SEM). Amounts as in Fig 1.

each group of rats, the volumes of middle ear fluid and quantities of albumin in the fluid recovered during each time interval were averaged and analyzed by two-way repeated-measures analysis of variance; $p < .05$ was considered statistically significant. Graphs were constructed from means and standard errors of means.

Middle Ear Histologic Analysis. Following the last fluid collection, the middle ear was perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS). The rat was subsequently sacrificed painlessly with an intracardiac injection of potassium chloride, and the middle ear was dissected free and placed in 4% paraformaldehyde in PBS.

After separating the tympanic bulla from the cochlea, a 0.4-mm-wide by 4-mm-long strip of middle ear mucosa and periosteum, extending between the tympanic membrane and the eustachian tube opening into the middle ear, was meticulously removed from the underlying bone with a microscalpel. During this procedure, extreme care was taken to avoid disrupting the neutrophil-impregnated fibrinous exudate overlying the mucosa. This strip of tissue was dehydrated in a series of increasingly concentrated alcohol solutions and then embedded in a paraffin block. Eight-micron cross sections of this tissue strip were cut with a microtome and mounted on slides. Sections approximately 220 μ m from the eustachian tube opening were stained with hematoxylin and eosin.

The stained sections were used to quantify the leukocytes in the middle ear mucosa and overlying

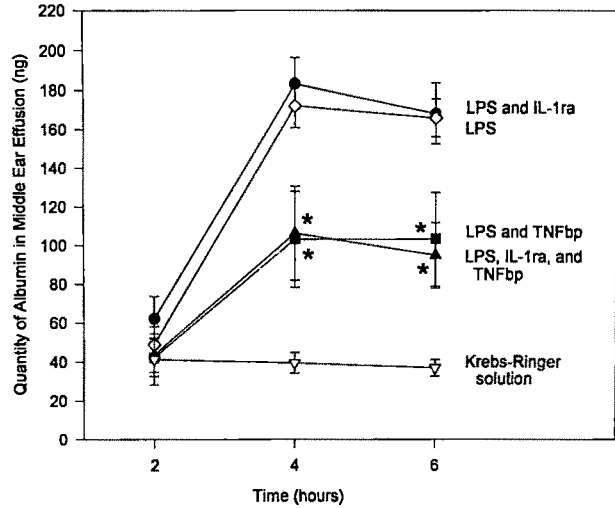


Fig 3. Mean quantity of albumin in fluid collected from middle ears every 2 hours over 6-hour period. Middle ears were exposed to either KR; LPS; LPS and IL-1ra; LPS and TNFbp; or LPS, IL-1ra, and TNFbp. Asterisk designates mean quantities of albumin that were significantly different from mean quantities of group whose ears were exposed to LPS alone. Error bars represent SEM. Amounts as in Fig 1.

exudate. One section from each rat middle ear was randomly chosen. The thickest region in which both mucosa and exudate were clearly defined was identified. In this region, the leukocytes in a 190- μ m-wide section of mucosa and exudate were counted. The numbers of leukocytes in two contiguous 190- μ m sections were also counted. These three counts were then averaged.

The mean leukocyte counts for all of the rats in a group were averaged and analyzed by one-way analysis of variance; $p < .05$ was considered statistically significant. Graphs were constructed from means and standard errors of the means.

This study complied with *The Principles of Laboratory Animal Care*, formulated by the National Society for Medical Research, and the *Guide for the Care and Use of Laboratory Animals*, prepared by the National Academy of Sciences and published by the National Institutes of Health.

RESULTS

Volume of Middle Ear Fluid. In the control group, the mean volumes of middle ear fluid (Fig 2) collected at the end of the first, second, and third 2-hour intervals were not significantly different. In all experimental groups, the mean volumes of fluid recovered at the end of the first interval were not significantly different from the corresponding control volume. In contrast, the mean volumes of fluid obtained after the second and third intervals for all experimental groups were significantly greater than the corre-

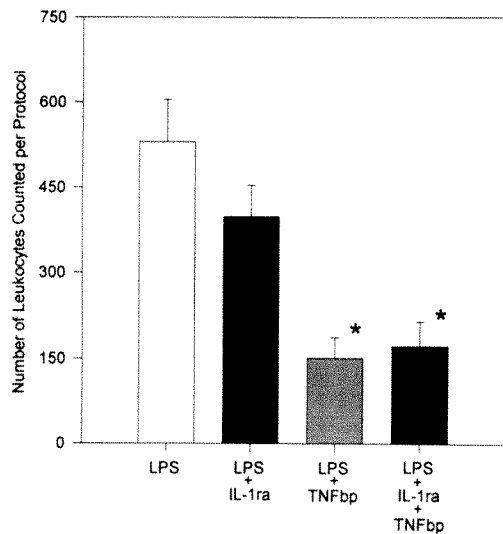


Fig 4. Mean number of leukocytes counted per protocol in histologic sections of periosteum, mucosa, and exudate from middle ears exposed to either LPS; LPS and IL-1ra; LPS and TNFbp; or LPS, IL-1ra, and TNFbp. Asterisk designates mean numbers of leukocytes that were significantly different from mean number counted from group whose ears were exposed to LPS alone. Error bars represent SEM. Amounts as in Fig 1.

sponding control volumes ($30.7 \pm 1.10 \mu\text{L}$ and $30.9 \pm 1.06 \mu\text{L}$). For the group exposed to LPS alone, these volumes were $44.3 \pm 1.44 \mu\text{L}$ and $44.6 \pm 1.07 \mu\text{L}$, respectively. These volumes were not significantly different from those of the group exposed to IL-1ra in addition to LPS. However, the mean volumes of fluid withdrawn at the end of the second and third intervals from the ears inoculated with either LPS and TNFbp ($35.5 \pm 0.95 \mu\text{L}$ and $37.3 \pm 1.36 \mu\text{L}$) or LPS and the combination of TNFbp and IL-1ra ($34.5 \pm 1.28 \mu\text{L}$ and $34.7 \pm 0.94 \mu\text{L}$) were significantly lower than the volumes from the LPS-only group, but were not significantly different from each other.

Quantity of Albumin in Middle Ear Fluid. In the control group, the mean quantities of albumin (Fig 3) in middle ear fluid collected at the end of the first, second, and third 2-hour intervals were not significantly different. In all experimental groups, the mean quantities of albumin recovered at the end of the first interval were not significantly different from the corresponding control quantity. In contrast, the mean quantities of albumin in the fluid obtained after the second and third intervals for all experimental groups were significantly greater than the corresponding control quantities ($39.6 \pm 5.30 \text{ ng}$ and $37.1 \pm 4.23 \text{ ng}$). For the group exposed to LPS alone, these quantities were $172.1 \pm 11.1 \text{ ng}$ and $166.1 \pm 9.71 \text{ ng}$, respectively. These quantities were not significantly different from those of the group exposed to IL-1ra as well as LPS. However, the mean quantities of albumin in the fluid withdrawn at the end of the second and third

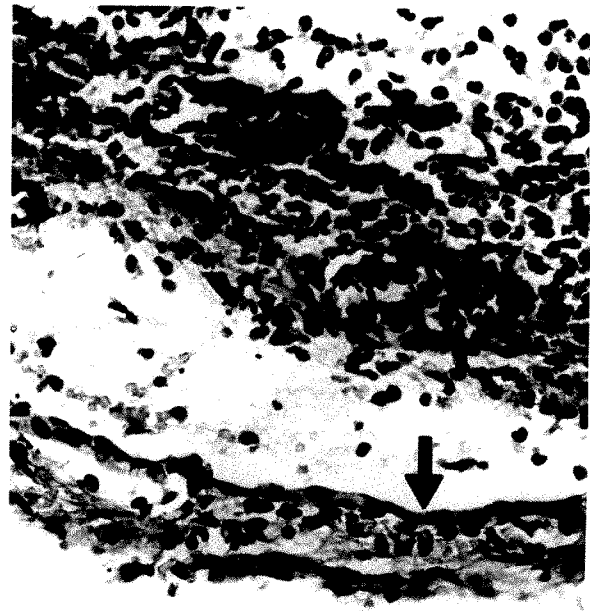


Fig 5. Representative cross section of middle ear mucosa and overlying neutrophil-impregnated exudate from middle ear exposed to LPS alone (original $\times 40$). Arrow — middle ear epithelium.

intervals from the ears inoculated with either LPS and TNFbp ($103.3 \pm 24.92 \text{ ng}$ and $103.4 \pm 24.4 \text{ ng}$) or LPS and the combination of TNFbp and IL-1ra ($106.4 \pm 24.4 \text{ ng}$ and $94.9 \pm 16.9 \text{ ng}$) were significantly lower than the quantities from the LPS-only group, but were not significantly different from each other.

Leukocyte Quantification. The numbers of leukocytes counted per protocol in the periosteum, mucosa, and exudate stripped from the control middle ears exposed to KR solution alone were less than 5 leukocytes per ear. The mean numbers of leukocytes counted from the experimental groups are graphed in Fig 4. For the ears exposed to LPS alone, the mean number was 529.7 ± 82.0 leukocytes (Fig 5). The mean number from the ears inoculated with IL-1ra and LPS was about 25% lower, but not significantly so. In contrast, the mean numbers in the tissue exposed to either LPS and TNFbp or LPS and the combination IL-1ra and TNFbp were significantly lower than the number of leukocytes in the middle ear sections taken from the LPS-only group, by approximately 70% (Fig 6). However, the numbers of leukocytes counted in both groups whose middle ears were injected with TNFbp were not significantly different.

DISCUSSION

Exposure of the rat middle ear to bacterial LPS induced an increase in both the volume and albumin content of the subsequent MEE compared to controls. These increases were first noted after 4 hours of LPS exposure and persisted through the sixth and final



Fig 6. Representative cross section of middle ear mucosa and overlying neutrophil-impregnated exudate from middle ear exposed to LPS, TNFbp, and IL-1ra (original $\times 40$). Arrow — middle ear epithelium.

hour of our experiment. Exposure of the ear to IL-1ra, in addition to LPS, had no effect on the volume and albumin content of the MEE induced by LPS. In contrast, TNFbp significantly suppressed the LPS-induced MEE, although not to control levels. The combination of TNFbp and IL-1ra also suppressed the LPS-induced MEE, but not more than TNFbp alone. These findings suggest that TNF, but not IL-1, is an active mediator of LPS-induced MEE.

Several studies have documented the presence of TNF and IL-1 in human MEE.¹⁶⁻¹⁸ However, only one attempt has been made to determine if TNF and IL-1 actively contribute to the development of MEE. Catanzaro et al¹⁹ transtympanically injected human recombinant IL-1 (rIL-1) and TNF (rTNF) into the middle ear of guinea pigs and discovered that rTNF, but not rIL-1, produced a cellular effusion (77% to 92% neutrophils) 24 hours after injection. To our knowledge, no other studies have tried to clarify the roles of IL-1 and TNF in MEE. Specifically, no studies have attempted to suppress LPS-induced MEE with TNF and IL-1 antagonists and thereby confirm that these cytokines actively mediate LPS-induced MEE.

Although the contributions of IL-1 and TNF to LPS-induced MEE are poorly understood, the roles of these cytokines in LPS-induced vascular extravasation have been investigated more extensively in tissues other than the middle ear, most prominently the lung, pleura, and skin.²²⁻²⁴ According to these studies, TNF, but not IL-1, is a mediator of LPS-induced vascular leakage.²²⁻²⁴ The TNF contributes to the delayed, but not immediate, phase of such leakage; the delayed phase begins approximately 2 hours after initial LPS exposure.²²

Our study was designed to assess the role of TNF and IL-1 in LPS-induced delayed vascular leakage in the middle ear. One milligram per milliliter of LPS was used to induce this leakage. This concentration of LPS, although greater than the minimum 10 $\mu\text{g/mL}$ required to induce an MEE in animal models^{8,10} and substantially greater than the maximum 512 ng/mL of endotoxin recovered from the effusions of patients with OME,⁵ was necessary to induce a quantifiable effusion over the 6-hour length of our study. However, this concentration was not large enough to trigger a significant increase in LPS-induced immediate permeability compared to controls. Consequently, changes in immediate permeability did not confound our study of the delayed phase of LPS-induced vascular extravasation.

To monitor changes in vascular extravasation in the middle ear, we measured the quantity of albumin in the effusions. Albumin has not been shown to be synthesized or secreted in the middle ear, and thus, the amount of albumin recovered from the middle ear is considered a reliable index of serum transudation.³ Moreover, albumin, in contrast to many larger serum proteins, is extravasated into the middle ear within the first 6 hours of a middle ear inflammatory response, the duration of our experiment.⁷

Although the primary purpose of our study was to evaluate the effect of TNFbp and IL-1ra on LPS-induced vascular extravasation into the middle ear, we also examined the effect of these antagonists on neutrophil migration, for the delayed phase of LPS-induced leakage is neutrophil-dependent.²⁵ In the lung, skin, and pleural cavity, neutrophil recruitment during the delayed phase is stimulated by both TNF and IL-1.^{22,26,27} However, only TNF-mediated neutrophil migration appears to contribute to LPS-induced delayed vascular extravasation in these tissues.²²⁻²⁴ In our study, we did not intend to investigate in exhaustive detail the role of TNF and IL-1 in neutrophil infiltration during the delayed phase of LPS-induced MEE. Instead, our goal was simply to corroborate that TNF, but not IL-1, is at least in part responsible for the neutrophil recruitment on which the delayed phase of vascular extravasation depends. To accomplish this, we counted neutrophils in a strip of middle ear mucosa and overlying exudate that was carefully removed from the bulla after fixation. We do not claim that this method is ideal for evaluating neutrophil migration. However, this simple and time-efficient method, which most importantly preserves the contiguity of the middle ear mucosa and overlying exudate during histologic processing in contrast to other techniques in which the mucosa is processed while attached to the bulla, served our purpose suffi-

ciently.

In essence, our results corroborate the aforementioned studies of vascular leakage in the lung, pleura, and skin following LPS challenge.²²⁻²⁴ We demonstrated that TNF is an active mediator of LPS-induced vascular extravasation in the middle ear. The mechanism by which TNF contributes to LPS-induced MEE is not clear; however, our finding that TNFbp suppresses neutrophil recruitment suggests that neutrophil migration is likely involved.

By antagonizing the activity of TNF, we were able to inhibit effusion development, although not completely. Assuming that we optimally blocked the activity of TNF, the inability to completely suppress LPS-induced leakage suggests that mediators other than TNF may contribute to LPS-induced MEE. According to our findings, IL-1 is not one of these mediators. Although this conclusion is in agreement with the previously mentioned studies,²²⁻²⁴ our finding that IL-1ra did not significantly inhibit neutrophil migration was not.^{26,27} The inability of IL-1ra to suppress neutrophil recruitment may indicate that IL-1ra did not effectively antagonize the activity of IL-1 in our model. However, we believe that our human IL-1 antagonist effectively inhibited the activity of rat IL-1; the IL-1ra that we utilized was the same as that used by Ulich et al²⁶ and thus was rat-cross-reactive. Several explanations may account for the unexpected results. First, IL-1 may not play a significant role in LPS-induced neutrophil migration into the middle ear, unlike in the lung and pleural cavity.^{22,26,27} This explanation is supported by the finding of Catanzaro et al.¹⁹ They demonstrated that human recombinant IL-1 did not induce a cellular MEE in guinea pigs. Furthermore, our method of evaluat-

ing neutrophil migration may account for the apparent discrepancy for several reasons. We counted neutrophils at the end of our 6-hour experiment. According to studies of neutrophil migration in the rabbit pleural cavity, neutrophil counts at 6 hours may not optimally reflect the effects of IL-1 on neutrophil recruitment; IL-1 appears to stimulate neutrophil migration later than TNF, between 4 and 12 hours after LPS exposure, so for the most part this stimulation would not have occurred during our experiment.²² Finally, the variation in neutrophil counts within each experimental group was large. Consequently, the 25% difference in neutrophil counts between the ears inoculated with LPS alone and those with LPS and IL-1ra was not statistically significant, even though the difference may have been real. Clearly, further study is necessary to elucidate the role of IL-1 in LPS-induced neutrophil migration into the middle ear.

In conclusion, our study is the first to verify that TNF is an active mediator of LPS-induced MEE; we demonstrated that TNFbp, a natural human cytokine antagonist, suppresses LPS-induced MEE. Therefore, TNFbp may represent a novel approach to the treatment of OME.

At present, the only widely accepted medical treatment for OME is antibiotic therapy. However, antibiotics do not benefit all children, and consequently, many children require tympanostomy tube insertion for OME. We believe that TNFbp may represent an alternative to surgery for the treatment of OME that is unresponsive to low-cost, low-risk antibiotics. Unquestionably, further study of TNFbp and the role of TNF in OME is warranted.

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PEDIATRIC OTORHINOLARYNGOLOGY

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REVERSIBLE SENSORINEURAL HEARING LOSS AFTER RENAL TRANSPLANT IMMUNOSUPPRESSION WITH OKT3 (MUROMONAB-CD3)

CHRISTOPHER J. HARTNICK, MD

ALAN F. COHEN, MD

RICHARD V. SMITH, MD

BRONX, NEW YORK

Reversible and irreversible ototoxicity has been documented following the administration of various therapeutic agents. Reversible hearing loss is a known complication following the administration of quinine, salicylates and other nonsteroidal anti-inflammatory drugs, and erythromycin. We report a case of reversible hearing loss following OKT3 (murine monoclonal antibody CD3) administration. OKT3, a monoclonal antibody used as an immunosuppressant following cadaveric renal transplants, was associated with a transient sensorineural hearing loss that reversed following discontinuation of OKT3.

KEY WORDS — immunosuppression, OKT3, sensorineural hearing loss, transplant.

CASE REPORT

A 41-year-old man was admitted to the hospital for acute rejection of a renal transplant and started on a daily regimen of 5 mg OKT3 (murine monoclonal antibody CD3; muromonab-CD3) four times per day. His past medical history was significant for right renal agenesis and a left megaureter that necessitated a cadaveric renal transplant 1 month prior to the hearing loss. On admission, the patient had no subjective hearing loss, nor did he have a history of hearing loss, tinnitus, or vertigo. He noted difficulty hearing,

poor speech discrimination, and an acute-onset, continuous, nonpulsatile tinnitus approximately 36 hours after initiating OKT3 treatment. He denied any sensation of nausea or vertigo. On physical examination, the patient was found to be afebrile, and with a regular heart rate and rhythm. A head and neck physical examination yielded unremarkable findings, without nystagmus or otologic findings. The tinnitus slowly worsened over the ensuing 3 days. An audiogram on the third day of administration of OKT3 revealed a bilateral, mildly down-sloping, sensorineural hear-

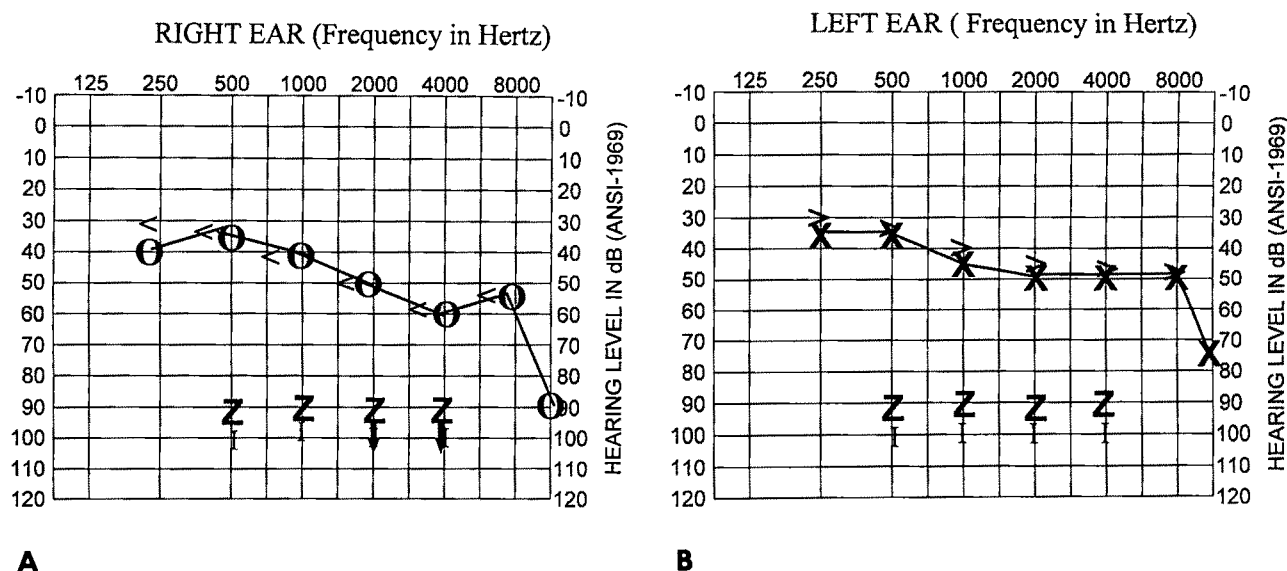


Fig 1. Pure tone audiograms representing acute onset of sensorineural hearing loss following administration of OKT3. Accompanying tympanograms for these audiograms were normal. For air conduction, O — right ear, X — left ear, Z — contralateral acoustic reflex, I — ipsilateral acoustic reflex. For bone conduction, < — right ear, > — left ear. A) Right ear. Speech threshold 30 dB, speech discrimination 92%. B) Left ear. Speech threshold 35 dB, speech discrimination 88%.

From the Department of Otolaryngology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, New York.

CORRESPONDENCE — Richard V. Smith, MD, Dept of Otolaryngology, 111 E 210th St, Bronx, NY 10467-2490.

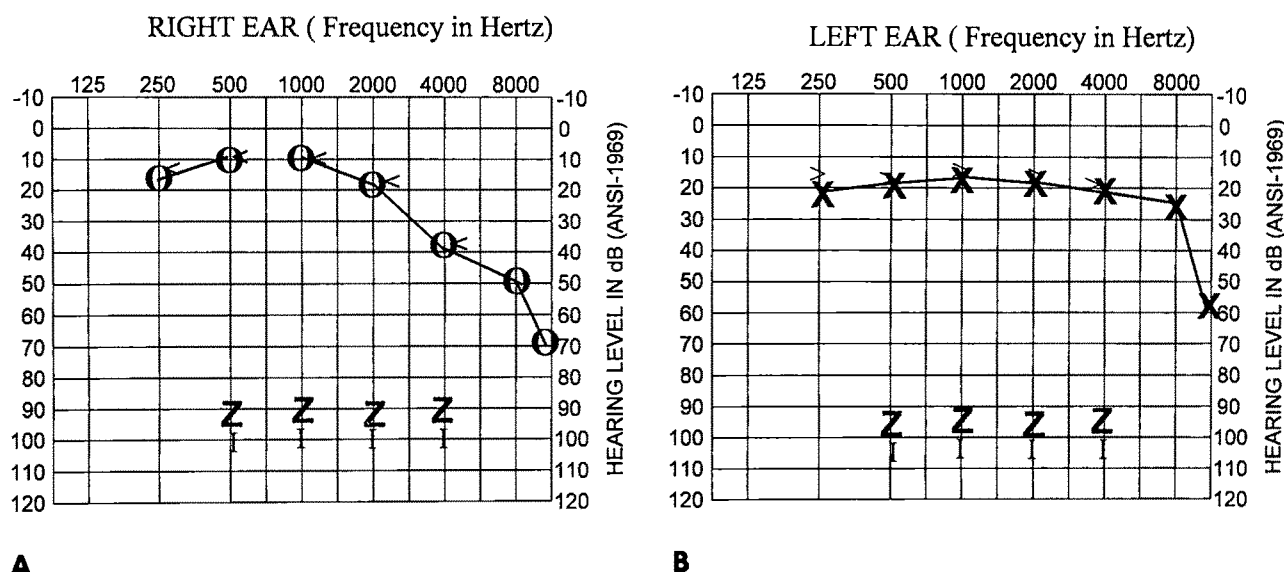


Fig 2. Pure tone audiograms representing subsequent resolution of hearing loss 10 days after discontinuation of OKT3. Accompanying tympanograms for these audiograms were normal. For air conduction, O — right ear, X — left ear, Z — contralateral acoustic reflex, I — ipsilateral acoustic reflex. For bone conduction, < — right ear, > — left ear. A) Right ear. Speech threshold 15 dB, speech discrimination 100%. B) Left ear. Speech threshold 10 dB, speech discrimination 96%.

ing loss (Fig 1). Speech thresholds were 30 dB and 35 dB for the right and left ear, with discriminations of 92% and 88%, respectively. The patient's tympanograms and acoustic reflexes were normal.

The patient was treated with OKT3 for a total of 8 days, until it was felt that the OKT3 could be safely discontinued. During this time, the patient was closely observed for progression of the auditory findings, and no change was noted. The patient was discharged home on the same medications as those he had taken previously. Over the next several days his hearing slowly improved, although the tinnitus persisted. A follow-up audiogram 10 days after the discontinuation of OKT3 showed marked improvement (Fig 2): the audiograms for the right and left ears showed essentially normal hearing up to 2,000 Hz, with a down-sloping, high-frequency sensorineural hearing loss. The speech thresholds and discriminations were 15 dB and 100% for the right ear and 10 dB and 96% for the left ear. He has since noted slow improvement of the tinnitus.

DISCUSSION

Reversible ototoxicity has been demonstrated after the administration of various pharmacologic agents, including salicylates¹ and erythromycin.² Salicylate-induced ototoxicity has been the most extensively studied model for reversible hearing loss, and seems to be multifactorial. While there is no obvious morphologic cochlear damage, salicylates seem to alter the electrophysiologic milieu of the outer hair cells. They also seem to exert an effect, via arachidonic acid metabolites, on cochlear blood flow.

Also to be considered in the differential diagnosis

of any case of sudden hearing loss is the possibility of trauma, syphilis, a postviral syndrome, an autoimmune phenomenon, a perilymphatic fistula, or a transient ischemic event. In the case discussed above, the differential is focused and narrowed by the history antecedent to the presenting symptoms. The patient had no recent history of trauma, nor any recent history that would predispose him to developing a perilymphatic fistula. The possibility of the patient's having a postviral, an autoimmune, or an ischemic-related hearing loss remains plausible; however, the patient's lack of other symptoms suggesting a prior viral cause, the time course associating the advent of hearing loss with the administration of OKT3, and the resolution of hearing loss with cessation of OKT3 strongly point toward the role of OKT3 in this case of reversible hearing loss.

OKT3 is a murine monoclonal antibody used in the treatment of acute rejection of renal allografts. This antibody reacts with the CD3 portion of a surface molecule on thymocytes and mature human T-cells.³ Once bound to OKT3, the CD3+ cells are opsonized, and subsequently removed by the reticuloendothelial system.⁴ OKT3 has been found to significantly reduce the rate of renal transplant rejection⁵; however, the use of OKT3 has been hampered by a constellation of side effects that range from pruritus, rashes, headaches, and diarrhea to hypotension, aseptic meningitis, seizures, and anaphylaxis.⁶ Reported aural side effects include auditory hallucinations and otitis media (secondary to increased vascular permeability), but there has been no documentation in the literature to date of OKT3's producing sensorineural

hearing loss. The current patient had no prior history of hearing loss or other auditory complaints. He was taking furosemide as a part of the initial posttransplant regimen; however, he noted no hearing loss during the month he was on the drug prior to the hearing loss. Moreover, once the course of OKT3 had been completed, the patient continued taking the same dose of furosemide. The only variable that marked the transient loss of hearing and the advent of tinnitus was the administration of OKT3.

The pathophysiology of this case of transient sensorineural hearing loss is unknown. One possibility hinges on the association of OKT3's so-called "first-dose" reaction with the release of several cytokines.⁷ The effect of various cytokines and neurotrophins on the organ of Corti has been well documented.⁸ Further investigations into the effects of OKT3 on the inner and outer hair cells, as well as on the spiral

ganglia neurons, might be insightful. Another possible explanation for the untoward effects of OKT3 on the auditory system is suggested by the increased vascular permeability following administration of OKT3, which can predispose a patient to developing otitis media. It may well be that such vascular changes alter the environment of the inner as well as the middle ear. Whatever the exact explanation may be, the effect seems to be reversible, as discontinuation of OKT3 led to a resolution of hearing in this patient.

To date, the patient still has sensorineural hearing loss at frequencies higher than the normal spoken frequencies and some degree of tinnitus. It remains to be seen whether the effects of OKT3 are fully reversible after its discontinuation. However, it is notable that the auditory side effect that is perhaps the most severe, namely, hearing loss, is transient in nature and appears to abate after the discontinuation of OKT3.

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AUDIOLOGIC FEATURES OF HEARING LOSS DUE TO THE 1,555 MUTATION OF MITOCHONDRIAL DNA

TAKASHI TSUIKI, MD

MORIOKA, JAPAN

KAZUO MURAI, MD

MORIOKA, JAPAN

KEN KITAMURA, MD

MINAMIKAWACHI, JAPAN

SEIKO MURAI, MD

MORIOKA, JAPAN

YUYA TAMAGAWA, MD

MINAMIKAWACHI, JAPAN

We proved a 1,555 mutation of mitochondrial DNA in one member of each of three families with familial streptomycin hearing loss, and report the pedigrees and audiologic features. DNA was extracted by the standard method. The 1,555 A to G mutation was identified in all three patients and confirmed by direct sequencing of the polymerase chain reaction products by a cycle sequencing method. On audiograms, the hearing loss was sensorineural, bilateral, and symmetric, showing a high-tone loss or a profound loss particularly in the high-tone range, and the "symmetry law" of Langenbeck was applicable. The superimposed audiograms of members of one family did not cross themselves, proving the applicability of the "never-cross principle of audiograms."

KEY WORDS — audiologic feature, mitochondrial DNA, sensorineural hearing loss, streptomycin.

INTRODUCTION

The characteristics of sensorineural hearing loss induced by dihydrostreptomycin (DHSM) can be summarized by the following three points: hearing loss may occur even with a very small dosage of DHSM; the loss of hearing may develop or progress after discontinuation of drug administration; and the development or extent of hearing loss varies greatly according to individual differences. Therefore, these characteristics led us to believe that this hearing loss does not occur as a result of an overdosage of the drug, but that it may occur as a result of individual susceptibility.¹

Cases of hearing loss appearing in brothers were reported by Tsuike¹ and Prazic et al.² Then Tsuike and Murai³ reported on a family with several members developing hearing loss after receiving DHSM, and also several members developing hearing loss of endogenous origin of unknown cause without DHSM. Tsuike and Murai suggested that one form of predisposition to hearing loss is characterized by the vulnerability of the inner ear itself and that this may be transmitted by an autosomal dominant inheritance.

Johnsonbaugh et al.,⁴ Viljoen et al.,⁵ and Sun et al.⁶ reported several cases of this kind. In 1991, Hu et al.⁷ suggested that since many cases of familial streptomycin hearing loss were occurring by maternal transmission, mitochondrial inheritance must be the most

probable source. Furthermore, in 1993, Prezant et al.⁸ and Hutchin et al.⁹ reported that it had been caused by a nucleotide 1,555 mutation of mitochondrial DNA, and since then, only a few investigative reports¹⁰⁻¹² have been conducted on the 1,555 mutation. Thus, genetic studies of familial streptomycin hearing loss have progressed; however, there have not been sufficient audiologic studies.

Although Tsuike,¹ Prazic et al.,² Tsuike and Murai,³ and Johnsonbaugh et al.⁴ have reported audiograms of familial streptomycin hearing loss, audiograms show-

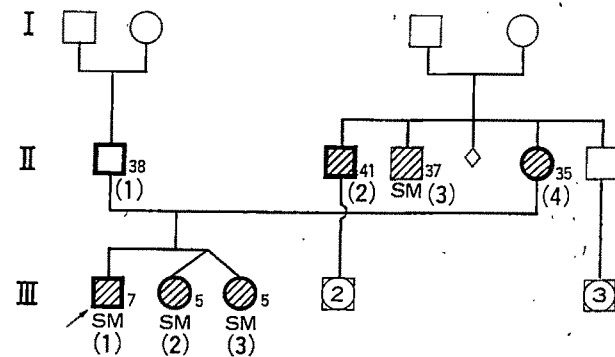


Fig 1. Pedigree of family A. For all pedigrees (Figs 1, 3, and 5), square — male; circle — female; shading — hearing loss; circle within square — male(s) and female(s); number inside symbol — number of siblings; heavier outline — examined; SM — dihydrostreptomycin given; small number to right of symbol — age; arrow — proband; diamond — abortion or stillborn; number in parentheses — identification number.

From the Department of Otolaryngology, Iwate Medical University (Tsuike, K. Murai), and the Department of Otolaryngology, Morioka Municipal Hospital (S. Murai), Morioka, and the Department of Otorhinolaryngology, Jichi Medical School, Minamikawachi (Kitamura, Tamagawa), Japan.

CORRESPONDENCE — Takashi Tsuike, MD, Dept of Otolaryngology, Iwate Medical University, 19-1 Uchimaru, Morioka 020, Japan.

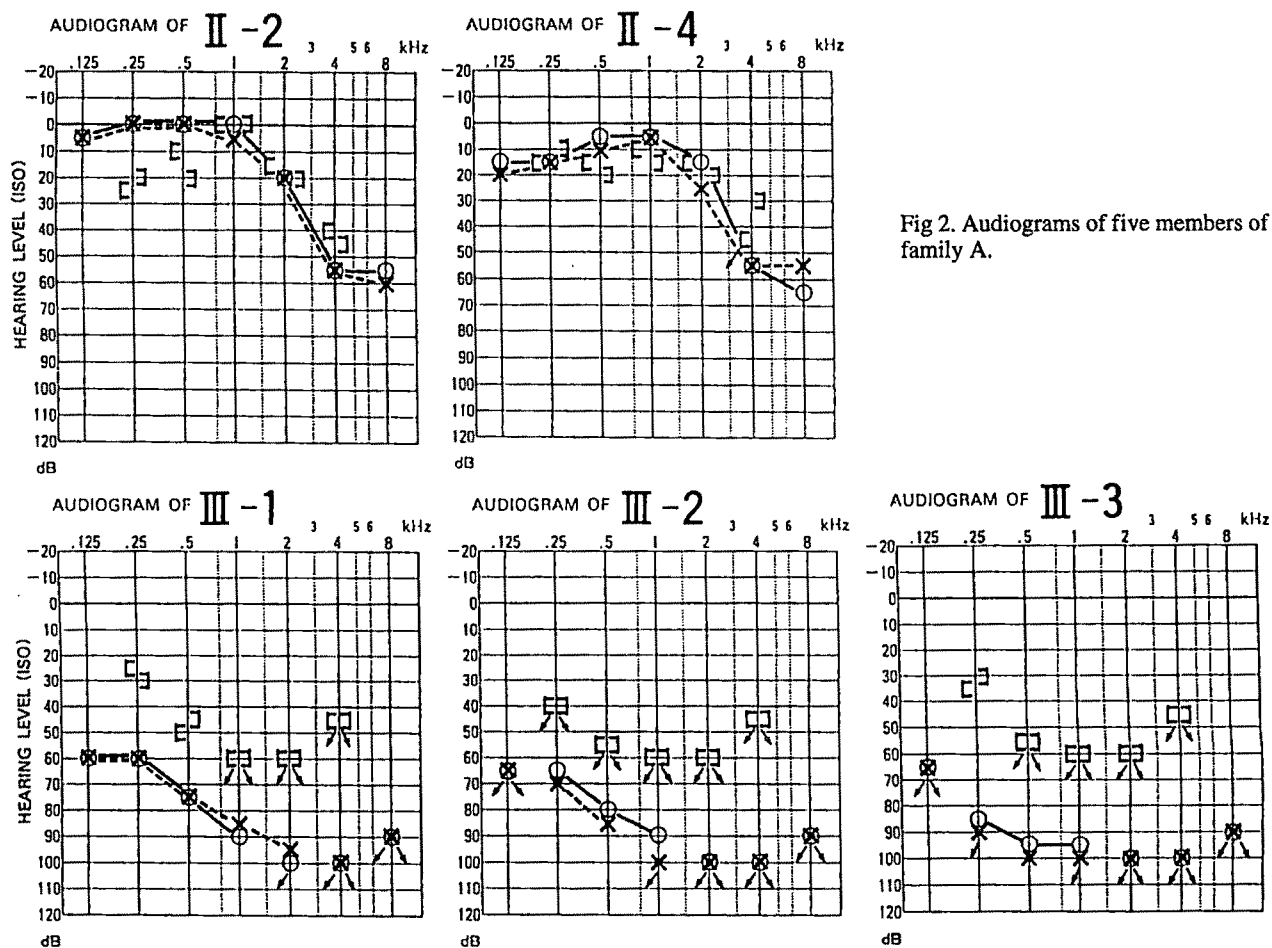


Fig 2. Audiograms of five members of family A.

ing cases of 1,555 mutation have not as yet been reported. We have proved the 1,555 mutation of mitochondrial DNA in one member of each of three families with familial streptomycin hearing loss; the pedigrees and audiograms are reported, and the audiologic features discussed.

CASES

Family A. The pedigree of family A is shown in Fig 1, and audiograms of five members (III-1, III-2, III-3, II-2, II-4) are shown in Fig 2.

Subject III-1 (proband, a 7-year-old boy) became

deaf following a DHSM intramuscular injection when he contracted a febrile illness at the age of 2 years 7 months.

Subjects III-2 and III-3 (twin sisters of proband, 5 years old) had received DHSM intramuscular injections several times since early childhood, and lost their useful hearing in early infancy.

Subject II-1 (proband's father, 38 years old) had no hearing loss, subjectively or audiometrically.

Subject II-2 (proband's uncle, 41 years old) had sensorineural hearing loss, but no significant features. No DHSM was given.

Subject II-3 (proband's uncle, 37 years old) was administered DHSM intramuscularly for spinal caries, and became profoundly deaf. Audiometry was not carried out.

Subject II-4 (proband's mother, 35 years old) was administered non-aminoglycoside antibiotics several times when she suffered from pyrexia. She had sensorineural hearing loss bilaterally.

Family B. The pedigree of family B is shown in Fig 3, and audiograms of three members (III-1, III-2, II-2) are shown in Fig 4.

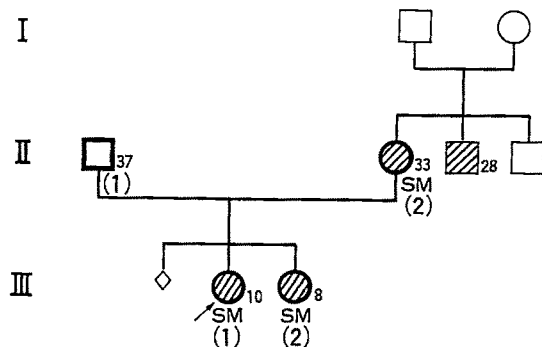


Fig 3. Pedigree of family B. See Fig 1 for symbols.

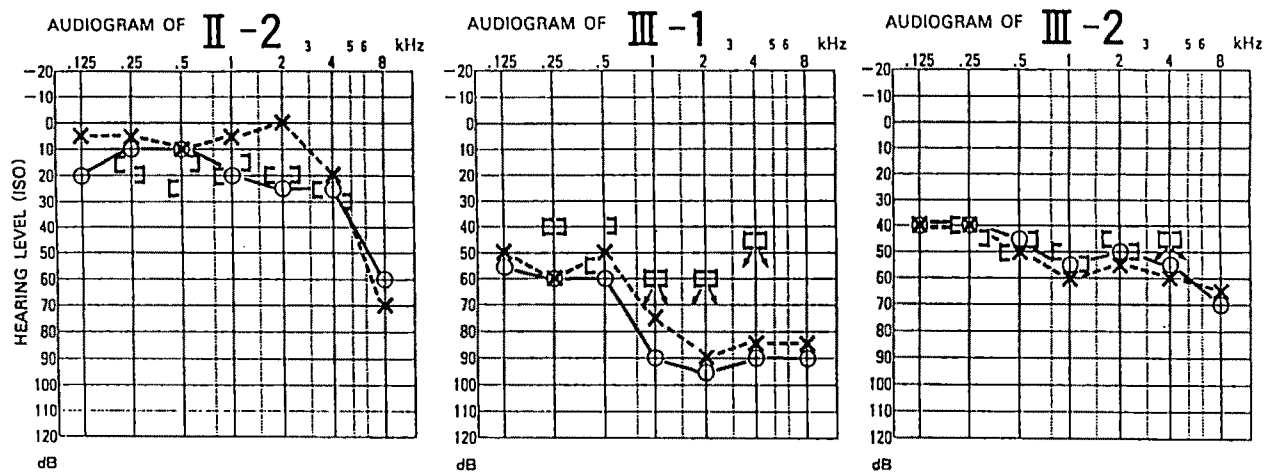


Fig 4. Audiograms of three members of family B.

Subject III-1 (proband, a girl 10 years old) showed evidence of hearing loss following a DHSM injection when she was suspected of having pulmonary tuberculosis at the age of 2.

Subject III-2 (proband's sister, 8 years old) had DHSM injection for cystitis at the age of 3, after which she became aware of the hearing loss.

Subject II-1 (proband's father, 37 years old) had no DHSM injections, and no complaint of any ear symptom. A pure tone audiogram showed normal hearing.

Subject II-2 (proband's mother, 33 years old), at the age of 20, had DHSM injection for suspected pulmonary tuberculosis, after which she complained of tinnitus and hearing loss.

Family C. The pedigree of family C is shown in Fig 5, and audiograms of six members (III-1, III-2, III-3, II-2, II-3, I-1) are shown in Fig 6.

Subject III-1 (proband, a girl 14 years old) had, at the age of 12, noticed difficulty hearing in both ears. No DHSM was given.

Subject III-2 (proband's sister, 11 years old), at the age of 11, was suspected of having a hearing loss. No DHSM was given.

Subject III-3 (proband's sister, 7 years old) had no problem in hearing subjectively. A high-tone hearing loss was found on an audiogram. There was no specific cause for hearing loss.

Subject II-1 (proband's father, 44 years old) had no subjective complaint concerning ear symptoms, and no hearing loss was recorded on the audiogram. No DHSM had been given.

Subject II-2 (proband's mother, 39 years old), at the age of 18, had been given DHSM by injection for pleuritis; subsequently, hearing loss and tinnitus developed.

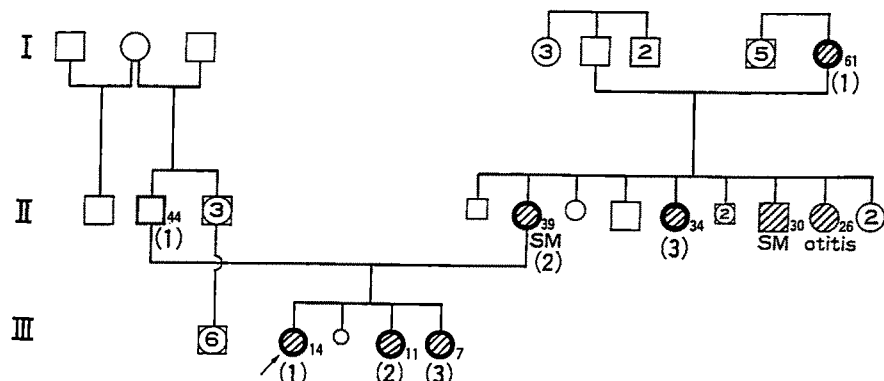
Subject II-3 (proband's aunt, 34 years old) had hearing difficulty for several years with no causative factor identified. No DHSM had been given.

Subject I-1 (proband's grandmother, 61 years old) had no subjective difficulty in hearing. There was a high-tone loss on her audiogram. No DHSM was given.

The pedigrees of the three families shown in Figs 1, 3, and 5 have been characterized by maternal transmission.

The audiograms of 14 subjects shown in Figs 2, 4,

Fig 5. Pedigree of family C. See Fig 1 for symbols.



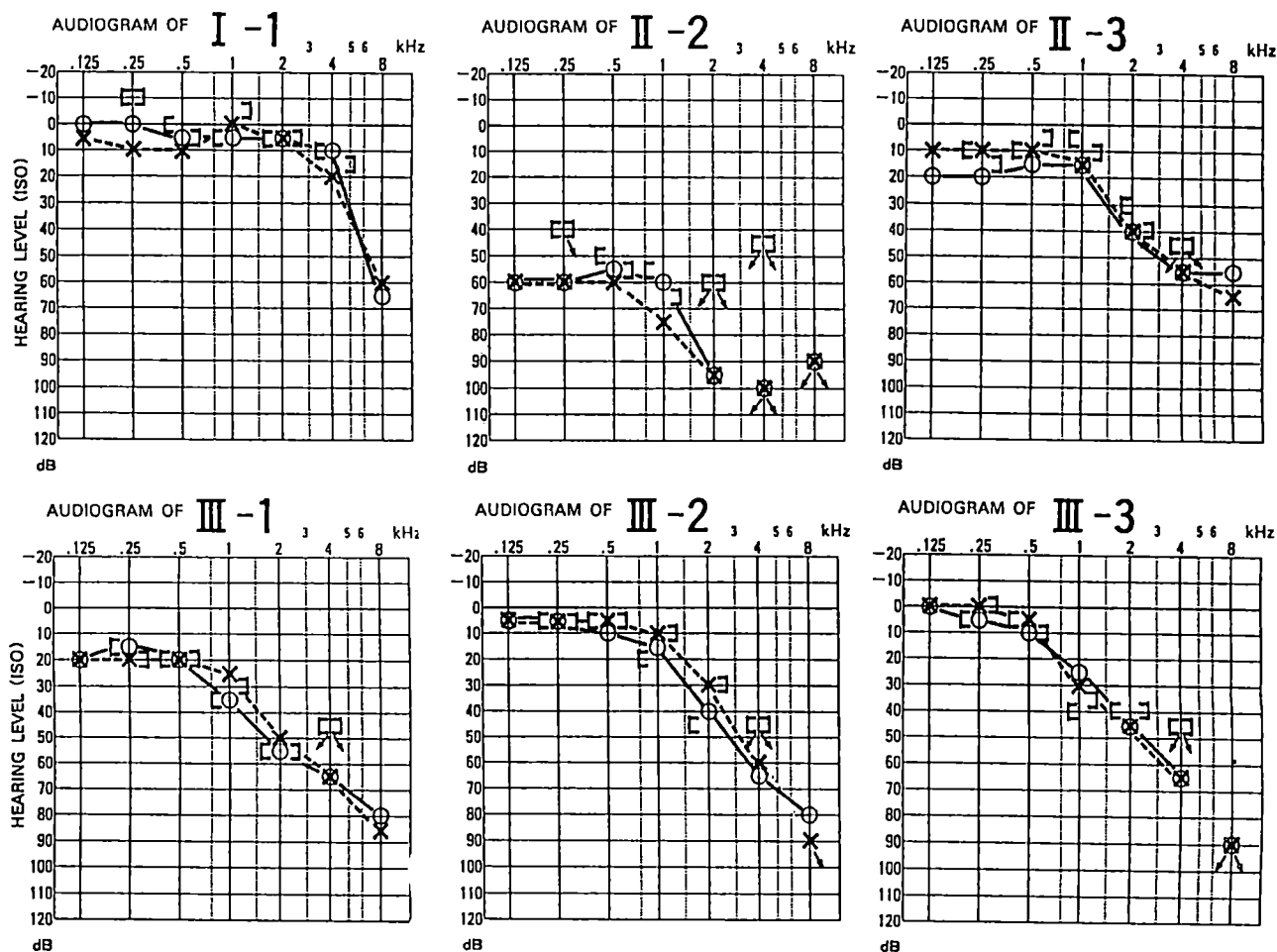


Fig 6. Audiograms of six members of family C.

and 6 have the following features in common: 1) bilateral hearing loss, 2) sensorineural hearing loss, 3) high-tone loss or profound loss particularly at the high-tone range, and 4) right and left audiograms that are almost the same.

MITOCHONDRIAL DNA STUDIES

Blood samples were obtained from patient III-3 of family A, subject III-1 from family B, and subject III-1 from family C after informed consent. DNA was extracted by the standard method. Mitochondrial DNA fragments encompassing position 1,555 were amplified with the polymerase chain reaction (PCR) from total DNA. The amplified products were digested with the endonuclease *BsmA* I. The 1,555 A to G mutation results in the loss of the *BsmA* I restriction site,^{8,11} thus providing a simple method for detecting the mutation. The digested mitochondrial DNA fragments were electrophoresed, stained with ethidium bromide, and photographed. The mutation was identified in all three subjects and confirmed by direct sequencing of the PCR products via a cycle sequencing method. Figure 7 illustrates the sequences

encompassing position 1,555 from a normal control and one subject, showing the 1,555 A to G mutation with no evidence of the coexistence of wild type and mutant genes in the subject. The other two subjects also appeared to be homoplasmic for the 1,555 A to G mutation (data not shown).

DISCUSSION

In 1936, Langenbeck¹³ advocated the "symmetry law of hereditary hearing loss." This means that the residual hearing is identical for both ears in genetically determined hearing loss. He said it can be a touchstone for the diagnosis of genetic hearing loss. In this study, all cases in which hearing disturbances had been observed were bilaterally affected and had almost identical audiograms of both ears. These results are applicable to Langenbeck's symmetry law. Tsuiki¹⁴ found that when all audiograms of the members of families with hereditary hearing loss were drawn in one chart, by overlapping them one could see that the audiograms coincided with each other, or never crossed each other. The phenomenon was called the "never-cross principle of audiograms." Tsuiki

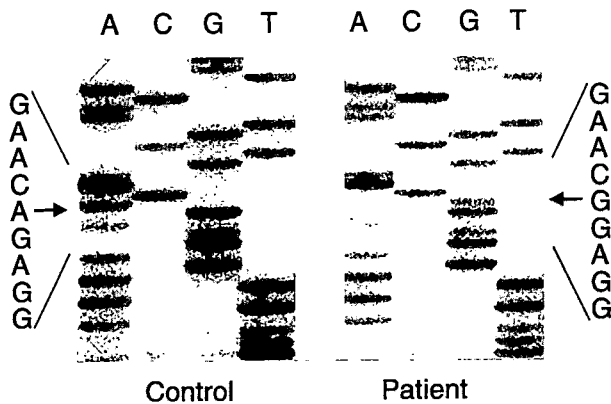


Fig 7. Mitochondrial DNA sequences surrounding position 1,555 from normal control (left) and patient III-3 from family A (right). Amplified DNA fragments were directly sequenced. Homoplasmic A to G mutation was demonstrated in patient.

explained this principle in the following way: the time-dependent change in the audiogram of a person with hereditary hearing loss might indicate a time-dependent progression that is characterized by a genetic code specific to the disease. If we assume that the time-dependent progression is always an increase and never a decrease in hearing loss, we can expect that all of the person's audiograms drawn superimposed would coincide with each other, or never cross each other. Furthermore, this expectation can be extended to the audiograms of different persons in the family, because the individual difference may simply reflect a time-dependent change in hearing loss characterized by the same genetic code. Such an expectation as described can be used as a working hypothesis, and called the "never-cross principle of audio-

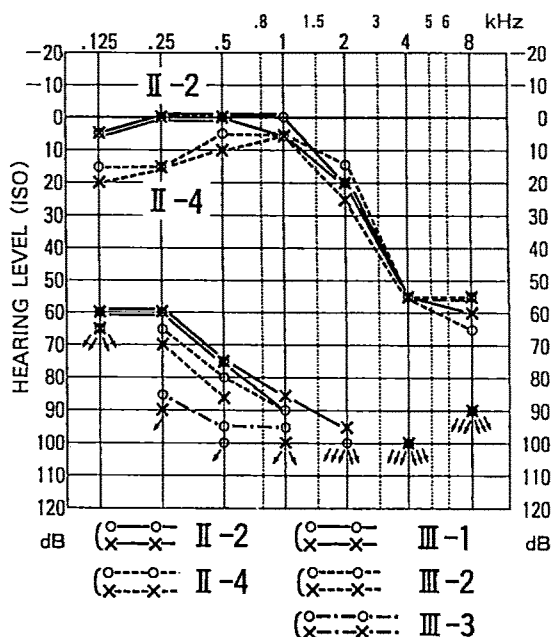


Fig 8. Superimposed audiograms of five members of family A.

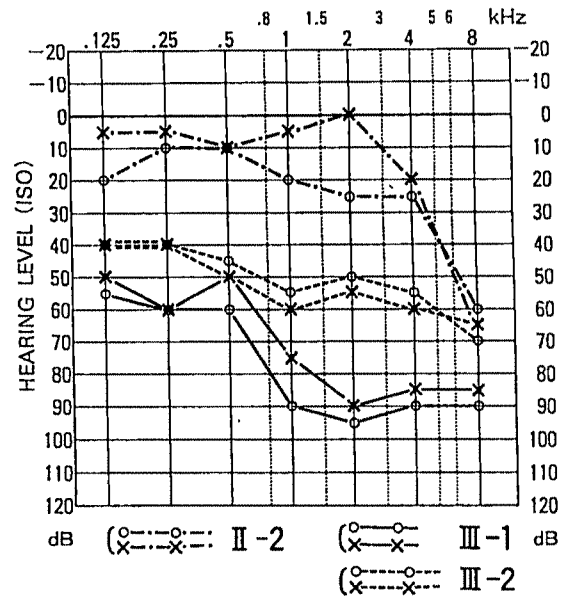


Fig 9. Superimposed audiograms of three members of family B.

grams" in a family with hereditary hearing loss. In this study, audiograms of members of these three families drawn superimposed in one chart do not cross (Figs 8-10). The above results show that hearing loss due to the mitochondrial DNA 1,555 mutations does not differ significantly from familial progressive sensorineural hearing loss, which is traditionally supposed to be caused by a well-known autosomal dominant inheritance.

In the past, hereditary hearing loss not associated with abnormalities in any other parts of the body was divided into two types: one is congenital deafness,

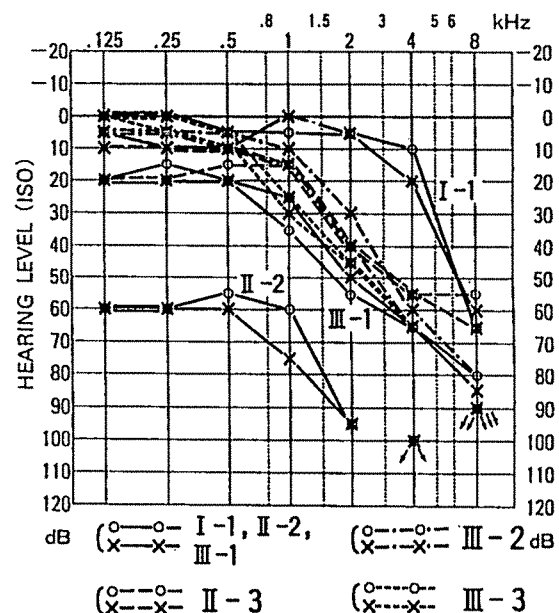


Fig 10. Superimposed audiograms of six members of family C.

appearing under the form of autosomal recessive inheritance, and the other is familial progressive sensorineural hearing loss, taking the appearance of autosomal dominant inheritance. The latter presents no hearing disturbance at birth as a rule, but it develops and progresses in early childhood, in adolescence, or at puberty. The audiograms of patients having this disease usually satisfy both the "symmetry law" and the "never-cross principle." The symptoms of the cases presented in this paper correspond to those of cases of classic familial progressive hearing loss. This leads us to assume that they would have something in common in the developmental process.

Recent reports seem to suggest that the 1,555 mutation of mitochondrial DNA has a particular susceptibility to aminoglycosides.¹⁰⁻¹² However, several cases of hearing loss not caused by aminoglycosides, like cases in this report (family A, subjects II-4 and II-2; and family C, subjects III-1, III-2, III-3, II-3, and I-1), were included. In addition, the same mitochondrial DNA mutation was found in a nonfamilial case of bilateral sensorineural hearing loss of unknown cause.¹⁵ In this respect, it seems that it is more judicious to think of this DNA mutation as a predisposition to hearing loss, sometimes displaying a particular predisposition with DHSM, rather than a susceptibility to aminoglycosides in general. The cases of familial hearing loss induced by aminoglycosides that we found were all caused by DHSM, and cases of familial deafness induced by other aminoglycosides, such as the original streptomycin, kanamycin, and gentamicin, etc, were not encountered. Consequently, this susceptibility should be

considered as one to DHSM, and it is not precise to refer to it as a susceptibility to aminoglycosides.

SUMMARY AND CONCLUSION

Three families having familial streptomycin-induced hearing loss, in which a 1,555 mutation of mitochondrial DNA was proved, are reported. Each family's pedigree and audiograms of most of the members with hearing loss are shown. The pedigrees presented the appearance of maternal transmission, and the hearing loss cases of the pedigree involved one without streptomycin. The audiograms showed the following features.

1. The hearing losses were sensorineural and bilateral.
2. The audiograms showed a high-tone loss or a profound loss particularly in the high-tone range.
3. The audiograms indicated left and right symmetric loss, and were applicable to the "symmetry law of Langenbeck."¹³
4. When audiograms of members of a family were drawn in a single chart superimposed, they did not cross between themselves, proving the applicability of the "never-cross principle of audiograms."¹⁴

This genetically determined susceptibility to hearing loss is not a susceptibility to aminoglycosides in general, but only to DHSM, and furthermore, it is more precise to think of it as a susceptibility to hearing loss itself.

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ANAEROBES AND FUNGI IN CHRONIC SUPPURATIVE OTITIS MEDIA

A. OLU IBEKWE, FRCS

ZAIN AL SHAREEF, MD, FACH ARZT

ASHRAF BENAYAM, MBBS

TABUK, SAUDI ARABIA

Microbiology of 102 ears with chronic suppurative otitis media was studied for aerobes, anaerobes, and fungi. Forty-four percent were pure cultures, 33.3% were mixed, and 18.6% had no growth. Seventy-four percent were aerobes, 25% fungi, and only 0.9% anaerobes. *Pseudomonas aeruginosa* (22.5%) was the most common isolate, followed by *Staphylococcus aureus* and the *Aspergillus* species. The possible reasons for low yield of anaerobes and the pathogenic roles of anaerobes and fungi in chronic suppurative otitis media are discussed. It is advocated that in investigating pathogenic organisms in chronic suppurative otitis media, requests should include anaerobes and fungi.

KEY WORDS — anaerobes, chronic suppurative otitis media, fungi, microbiology.

INTRODUCTION

Chronic suppurative otitis media (CSOM), whether atticointral or tubotympanic disease, is associated with mixed bacterial flora. In the past, cultures from CSOM grew only aerobic organisms.¹⁻³ The isolation of anaerobic organisms from otogenic brain abscess⁴ and the fact that anaerobic organisms outnumber aerobic organisms in oral flora suggested that anaerobes are involved in middle ear infections.⁵ This idea led to the use of improved anaerobic bacteriologic techniques in the investigation of bacterial flora of CSOM. With these improved techniques, 20% to 50% of isolates in CSOM⁶⁻¹⁰ are likely to be anaerobic. In all these studies, no mention was made of fungi.¹⁻¹⁰ It has always been assumed that fungi do not play any significant role in CSOM. However, fungi have been isolated in CSOM, especially from hot, humid regions of the world.¹¹⁻¹³ We therefore decided to study the microbiology of CSOM with the aim of determining the various pathogens, especially anaerobes and fungi, and their role in CSOM.

MATERIALS AND METHODS

This prospective study was carried out between November 1992 and June 1995. Patients with active CSOM attending the outpatient clinic of the Otolaryngology Department of North West Armed Forces Hospital, Tabuk, Saudi Arabia, and seen by the first author (A.O.I.) were involved in the study. The samples were collected from the middle ear via an aural speculum with use of a Mini-Tip Culturette (collection and transport system) containing 0.5 mL of modified Stuart's medium prepared by Becton Dickinson and Company, Cockeysville, Md.

The contents of the transport medium are sodium glycerophosphate, sodium thioglycolate, calcium chloride dihydrate, and water. The medium is capable of sustaining growth of aerobes, anaerobes, and fungi. The samples were inoculated into blood agar with 5% sheep's blood, chocolate agar, and MacConkey's agar plates within 1 hour of collection. The plates were incubated under carbon dioxide and examined at 24 hours and 48 hours for aerobes. For anaerobes, the material was plated on trypticase soy agar with 5% defibrinated sheep's blood, prepared by Saudi Prepared Media Laboratory (SPML), Riyadh, Saudi Arabia, and kanamycin-vancomycin laked blood agar plates, also prepared by SPML. The swabs were then placed in cooked meat medium containing vitamin K, sodium chloride, heme, tripticasein, and dextrose and incubated in anaerobic jars for 72 hours. For fungi, the specimen was inoculated into Sabouraud's dextrose agar plates and incubated for 4 days. The identification and characterization of the isolates were based on their cultural, morphologic, and biochemical characteristics.

RESULTS

A total of 102 patients were studied. Thirty-two (31%) were children under the age of 12 years. There were 111 isolates: 49 (44%) were pure cultures, 34 (33.3%) were mixed, and in 19 (18.6%) no growths were recorded.

Table 1 shows the various isolates. Eighty-two (74%) were aerobes, 28 (25%) were fungi, and only 1 (0.9%) was an anaerobe. *Pseudomonas aeruginosa*, in 25 (22.5%), was the most common bacterial isolate, followed by *Staphylococcus aureus* in 23

From the Department of Otolaryngology-Head and Neck Surgery, North West Armed Forces Hospital, Tabuk, Saudi Arabia.

CORRESPONDENCE — A. Olu Ibekwe, FRCS, Dept of ENT, North West Armed Forces Hospital, PO Box 100, Tabuk, Saudi Arabia.

TABLE 1. MICROBIOLOGIC ISOLATES FROM 102 EARS WITH CHRONIC SUPPURATIVE OTITIS MEDIA

Isolates	Total 111 Isolates		Pure Cultures 44%	
	No.	%	No.	%
<i>Pseudomonas aeruginosa</i>	25	22.5	21	18.9
<i>Staphylococcus aureus</i>	23	20.7	12	10.8
<i>Streptococcus pneumoniae</i>	12	10.8	1	0.9
<i>Haemophilus influenzae</i>	7	6.3		
<i>Proteus mirabilis</i>	4	3.6	3	2.7
<i>Klebsiella</i>	3	2.7		
Enterobacteriaceae	4	3.6		
<i>Acinetobacter calcoaceticus</i>	2	1.8		
<i>Serratia ficaria</i>	1	0.9		
<i>Bacteroides</i> sp (anaerobe)	1	0.9		
<i>Aspergillus</i> (fungus)	19	17.1	9	8.1
<i>Candida</i> (fungus)	9	8.1	3	2.7
No growth	19/102	18.6		
Total	111		49	44

Total number of patients was 102 (32 children were under 12 years of age).

(20.7%); there were fungi in 28 (25.2%). *Aspergillus* species (19 or 17.1%) made up the bulk of the fungi, and the rest (9 or 8.1%) were *Candida*.

DISCUSSION

The aerobic microbes isolated in this study correlate well with those in other studies, as illustrated in Table 2.^{11,14-18} *Pseudomonas aeruginosa* was the most common microbe in most of the studies in Table 2, except those of Jokipii et al¹⁴ and Ojala et al,¹⁵ in which *S aureus* was the most common. In children, *S aureus* is usually the most predominant organism, probably because most CSOM in children follows poorly treated acute suppurative otitis media, in which *S aureus* is the most common pathogenic organism.¹¹⁻¹⁵

It is generally accepted that anaerobes make up 20% to 50% of isolates in CSOM.⁶⁻¹⁰ This high yield can be attributed to improved techniques of collection, transportation, and inoculation of specimens.⁵

Our study did not confirm this finding. We only cultured 1 anaerobe from 102 specimens. An attempt will be made to find the cause for this poor yield.

Our specimens were collected from the middle ear through an aural speculum. We did not try to sterilize the ear canal, as advocated by Brook,⁵ the reason being that other studies have shown that external ear cultures correlated well with middle ear cultures.^{19,20} Our specimens were transported in commercially available transport media that allow for survival of anaerobic organisms. However, most of our patients were referred from primary health care physicians who first treated these patients with both systemic and topical antibiotics. The most common systemic antibiotics used were amoxicillin, Augmentin, and second-generation cephalosporins. The topical antibiotic was gentamicin ear drops. It is, therefore, possible that by the time these patients were seen, most of the anaerobes had been eliminated by antibiotic therapy. Another reason could be that perhaps we did not allow enough time for the slow-growing anaerobes to grow. Our cultures were for 72 hours, while Brook⁵ recommends 14 days.

Although 20% to 50% of CSOM can be attributed⁵ to anaerobes, their role in this infection is often questioned.²¹ However, observations by numerous workers suggest that anaerobes play a pathogenic role in CSOM. Anaerobes always grow in mixed cultures; they are usually grown with aerobes. Experiments show that when anaerobes or aerobes are inoculated separately, no inflammation is produced, but together, they produce intense inflammation with production of pus.²² This reaction is attributed to the synergistic relationship between aerobes and anaerobes.^{5,20} The recovery of anaerobes in otogenic intracranial complications also points to their pathogenicity.⁴ The production of β -lactamase by anaerobes and some aerobes and their ability to pass on their protective role to other organisms increase their pathogenicity in the mixed state.⁵

In our study, 25% of the isolates were fungi, espe-

TABLE 2. TYPES OF AEROBIC AND FUNGAL ISOLATES FROM VARIOUS STUDIES COMPARED WITH PRESENT STUDY

Authors	Year	No. of Ear Isolates	<i>Pseudomonas aeruginosa</i>	<i>Proteus</i> sp	<i>Escherichia coli</i>	<i>Klebsiella</i>	<i>Staphylococcus</i>	<i>Fungi</i>	
								<i>Aspergillus</i>	<i>Candida</i>
Palva and Hallstrom ¹⁶	1965	100	24.0%	8.0%	4.0%	ND	13.0%	ND	
Jokipii et al ¹⁴	1977	70	4.0%	8.0%	7.0%	ND	19.0%	ND	
Ojala et al ¹⁵	1981	806	19.0%	12.9%	6.8%	3.7%	22.0%	ND	
Ibekwe and Okafor ¹¹	1983	62	45.2%	12.9%	ND	ND	29.0%	4.3%	1.6%
Sugita et al ¹⁷	1981	62	7.8%	21.1%	ND	1.6%	6.3%	ND	ND
Fliss et al ¹⁸	1992	170	84.0%	Enteric gram-negative bacilli	32%	20.0%	ND	ND	
Present study		102	22.5%	3.6%	ND	2.7%	20.7%	17.1%	8.1%

ND — no data.

cially *Aspergillus* species (17.1%). Some studies from hot and humid environments also grew fungi.¹¹⁻¹³ It is interesting to note that studies from identical geographic areas were different in their culture of fungi. Dincer et al,¹³ in a study from Turkey, found 28.6% of the isolates were *Aspergillus* and *Candida*, while Erkan et al,²³ also from Turkey, did not report the growth of any fungi. Again, Ibekwe and Okafor,¹¹ in their study from Nigeria, reported about 6% of isolates to be due to fungi, while Rotimi et al,²⁴ also from Nigeria, did not report any fungal isolates. The possible explanation for this is that while one group looked for fungi, the other group did not. It is our belief that when looked for, fungi will invariably be isolated in ears with CSOM.

Fungal infections in otolaryngology are often associated with a decrease in immune function and are

often regarded as colonization rather than invasion and therefore as not requiring treatment. This assertion is far from the truth. Gregson and La Touche²⁵ suggested that active fungal infection, predominantly by species of *Aspergillus* or *Candida*, may be more common than is recognized and may explain failure to obtain dry ears in many cases of otorrhea. In CSOM, the development of otalgia, itching, and the presence of hyphae indicate the presence of fungi.¹¹ Recent reports of *Aspergillus* involving certain areas in otorhinolaryngology, eg, paranasal sinuses with intracranial complications,²⁶⁻²⁸ suggest that fungi can be pathogenic in ear infections as well. Fungi become pathogenic in an already inflamed mucosa, and unless they are treated when isolated in CSOM, a dry ear may not be achieved. We advocate that in investigating pathogenic organisms in CSOM, requests should include anaerobes and fungi.

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ELECTRICAL IMPEDANCE OF THE COCHLEAR IMPLANT LUBRICANTS HYALURONIC ACID, OXYCELLULOSE, AND GLYCERIN

LUCAS H. MENS, PHD
GODFRIED C. J. H. HOMBERGEN, PHD

THOM F. OOSTENDORP, PHD
PAUL VAN DEN BROEK, MD, PHD

NIJMEGEN, THE NETHERLANDS

Hyaluronic acid (Healon), oxycellulose (hydroxypropyl methylcellulose), and glycerin are lubricants used in cochlear implant surgery for atraumatic deep insertion of the electrode array into the scala tympani. The electrical impedances of these three lubricants were measured to assess possible effects on intraoperative evoked response measurements, such as the electrically evoked stapedius reflex and auditory brain stem response. The impedances of hyaluronic acid, oxycellulose, and saline were very similar and independent of frequency (20 Hz to 1 MHz). Glycerin had an excessively high impedance at low frequencies. A film of hyaluronic acid or oxycellulose around the electrode array immersed in saline did not have any measurable effect on the impedance; a film of glycerin resulted in a strongly reactive polarized layer. However, neither the far-field current spread nor the impedance between stimulated electrodes was affected by any of the lubricants applied as a thin film. This suggests that none of these lubricants affect intraoperative responses, when applied as a thin film.

KEY WORDS — cochlear implant, electrical resistivity, evoked responses, impedance, lubricative substances.

INTRODUCTION

In recent years, hyaluronic acid (Healon), oxycellulose (hydroxypropyl methylcellulose), and glycerin have been proposed as lubricants for cochlear implant surgery.^{1,2} These fluids serve to facilitate the atraumatic deep insertion of the cochlear implant electrode array into the scala tympani of the cochlea. Trauma to the spiral ligament and Reissner's membrane can occur easily, especially at the end of the basal turn,³ possibly damaging neural elements and causing secondary reactive osseous formation.⁴ Excessive mechanical resistance to the insertion due to obstructions may also damage the electrode array. It can be expected that deep insertion of the electrode array beyond the basal turn will result in more natural place-pitch mapping. Furthermore, incomplete insertion implies fewer usable electrodes and less recruitment of surviving spiral ganglion cells.² For the 22-electrode Cochlear Nucleus implant device, it has been established that when the apical electrodes are not used for stimulation, implant performance is poorer, on average, than when the medial or basal electrodes are not used.⁵ This finding underlines the need for deep insertion.

According to Donnelly et al,⁶ hyaluronic acid does not affect the hearing thresholds of implanted cats with normal hearing; in the same study, the use of hyaluronic acid enabled deeper insertion into 6 fresh

human temporal bones than in 22 patients implanted without the use of hyaluronic acid. Roland et al² found that an intracochlear injection of hyaluronic acid, glycerin, or oxycellulose did not have any effect on the spiral ganglion neurocyte count in guinea pigs 8 weeks after the injection. The dendrite and axon histology was also well preserved. In some animals, the acoustically evoked compound action potential was reduced in amplitude immediately after the injection of glycerin. Hyaluronic acid was the only substance that did not cause a pressure drop in the cochlear fluid (hydrops). All three substances were considered suitable for cochlear implantation, although oxycellulose was said to be nonmetabolized.

No data are available on the electrical properties of these lubricants. Many implant centers measure electrically elicited stapedius reflex and auditory brain stem thresholds in young children immediately after insertion of the electrode array. These thresholds are used as objective indications of the postoperative threshold and comfort levels needed for fitting the speech processor. Such measurements will be invalid if the lubricant alters the intracochlear electrical impedance such that the compliance voltage of the stimulator is reached, or if the current is directed away from the neural structures.

In this study, the impedances of hyaluronic acid, oxycellulose, and glycerin, both in pure form as well

From the Department of Otorhinolaryngology, University Hospital Nijmegen (Mens, Hombergen, van den Broek), and the Laboratory of Medical Physics and Biophysics, University of Nijmegen (Oostendorp), Nijmegen, the Netherlands.

CORRESPONDENCE — Lucas H. Mens, PhD, Dept of Otorhinolaryngology, University Hospital Nijmegen, PO Box 9101, 6500 HB Nijmegen, the Netherlands.

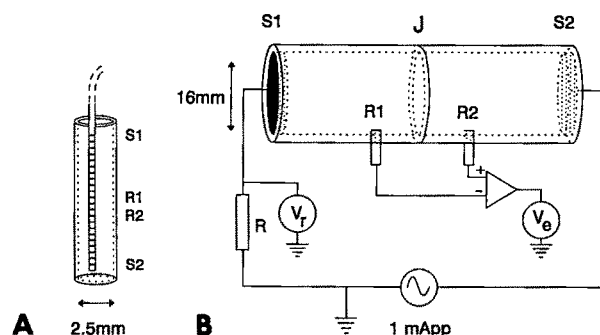


Fig 1. Test containers used for resistivity measurements. A) Test container A, small tube; Nucleus array was free-fitted. S1 and S2 are stimulated electrodes; R1 and R2 are electrodes used for registration. B) Test container B, Perspex cylinder in which specimen could be fitted at J. Illustrates four-terminal method. See text for abbreviations.

as in thin films, were compared to the impedance of saline.

METHODS

The 22 electrodes of the Nucleus Mini System 22 are bands (rings) of pure platinum, 0.3 mm wide and spaced at 0.75-mm intervals along the distal 17 mm of a silicone elastomer carrier. The electrode array tapers smoothly from a diameter of 0.6 mm at its widest part, where it enters the round window when implanted, to about 0.4 mm at the tip. The electrodes are numbered 1 to 22 from base to tip.

The impedance was measured by the four-terminal method,⁷ which eliminates electrode polarization as a contaminating factor. Two test containers were used (Fig 1).

Container A was a small cylindrical tube (radius 1.25 mm); an experimental version of the Nucleus Mini System 22 electrode array was fitted along its central axis. This device provided direct electrical access to the electrodes. The two outermost electrodes (Nos. 1 and 22; S1 and S2 in Fig 1A) were used to apply an excitation current. The potential difference between the two inner electrodes (Nos. 10 and 14; R1 and R2 in Fig 1A) was measured. Container B consisted of a Perspex cylinder (radius 8 mm) closed at both ends by silver chloride electrodes (S1 and S2), to which the current could be applied. Two small sensing electrodes (R1 and R2) were fitted into the wall of the container 12 mm from a central junction (J in Fig 1B). A specimen of material could be mounted at the junction.

In each container, the two outermost electrodes were used to induce a current of 1 mA (peak to peak) as established by measuring the potential difference V_r across a resistance R of 1 kilo-ohm ($k\Omega$; Fig 1B). The potential difference V_e across the sensing elec-

trodes was measured to yield the impedance Z . The resistivity ρ of the section of the container between the two inner electrodes was derived from

$$\rho = Z \times \frac{O}{d}$$

where O is the cross section of the container (minus that of the electrode array in the case of container A) and d is the distance between the sensing electrodes. The impedances were measured with a sinusoidal driving voltage in the frequency range from 20 Hz to 1 MHz. Phase differences were measured by displaying V_r and V_e simultaneously on an oscilloscope with a differential channel used for V_e . In addition to the resistivity measurements, the impedance between stimulated electrodes was derived with a sinusoidal driving current of 1 mA (peak to peak).

Measurements were taken in container A with a Nucleus Mini System 22 electrode array 1) immersed in undiluted lubricant and 2) covered with a film of lubricant immersed in saline (mimicking the surgical procedure) to study interfacial polarization. Interfacial polarization effects were also assessed by measuring across a piece of tissue paper soaked with the lubricant fitted at the central junction of container B.

The electrodes of the implant array that were not involved in the measurement were disconnected. However, the total area of the banded electrodes is considerable and may present a low-impedance path in container A. Control measurements in saline were therefore taken in container B.

Far-field current spread was measured by immersing the array into a shallow water tank filled with saline solution. The electrode array was positioned parallel to the line between the sensing electrodes; the array was positioned at one end of the tank, 12 cm away from the sensing electrodes at the other end. The sensing electrodes were 4 cm apart.

The hyaluronic acid was 10 mg/mL sodium hyaluronate (Healon) from Kabi Pharmacia. Oxycellulose was prepared as 1% hypromellose with 9 mg/mL sodium chloride and 0.5 mg/mL borax. Glycerin was 85% glycerol and 15% water with less than 0.001% residual elements. Saline was sodium chloride 0.9 g/L. Measurements were taken at 22°C and 37°C ($\pm 2^\circ\text{C}$).

RESULTS

The four-terminal method requires a homogeneous sample. Although the array was free-fitted in container A, with considerable deviations from the central axis, a uniform voltage drop was present in saline across all the electrodes 2 through 21, with a constant current between electrodes 1 and 22. This suggests

RESISTIVITY AT 10 KILOHERTZ

Temperature	Saline 0.9 g/L	Undiluted Hyaluronic Acid	Undiluted Glycerin	Undiluted Oxycellulose
22°C	59 ± 9	61 ± 9	(34 ± 5) × 10 ³	60 ± 9
37°C	44 ± 7	44 ± 7	(49 ± 7) × 10 ³	46 ± 7

Data are in $\Omega\cdot\text{cm}$, and represent value $\pm$ error margin based on estimated accuracy with which voltage and current readings were obtained.

that the requirement of homogeneity was satisfied. The large platinum-banded electrodes did have some effect on the resistivities measured. A resistivity of 59 $\Omega\cdot\text{cm}$ was measured in saline at 22°C at a frequency of 1 kHz when the implant was inserted into container A, compared to 64 $\Omega\cdot\text{cm}$ for saline measured in container B. Weast et al⁸ give a value of 61.1 $\Omega\cdot\text{cm}$ for the resistivity of saline at 22°C. These deviations were considered to be minor, and no correction was applied to the data obtained from container A.

The Table lists the resistivity values at 10 kHz for the undiluted lubricants and saline in container A.

In Fig 2, the resistivities of saline, hyaluronic acid, and oxycellulose are displayed as a function of frequency. This Figure shows that the resistivities were largely independent of the frequency up to about 500 kHz. Glycerin displayed a high-pass character across the frequency range applied in this study.

In repeated tests, the resistivity of glycerin was found to increase by about 1.5%/°C at 10 kHz, in contrast with a negative temperature coefficient for the other materials.

The application of a thin film of hyaluronic acid or oxycellulose around the electrode array immersed in saline (37°C) did not have any measurable effect on the impedance. A film of glycerin, however, resulted in an "effective resistivity" of 149, 80, and 58 $\Omega\cdot\text{cm}$ at 1, 10, and 100 kHz (compared to the 44 $\Omega\cdot\text{cm}$ for saline) and a phase angle of 180° at all the frequencies, which suggested interfacial polarization. Therefore, the volume conduction in container A with a film of glycerin was not homogeneous, and the resistivity could not be measured.

No fluid-fluid boundary effects were found for hyaluronic acid or oxycellulose when they were applied to a piece of tissue paper separating the two halves of container B. The application of glycerin, on the other hand, increased the magnitude of the impedance by about 30% at 1 kHz, by 15% at 10 kHz, and by 10% at 100 kHz.

The impedance between the stimulated electrodes

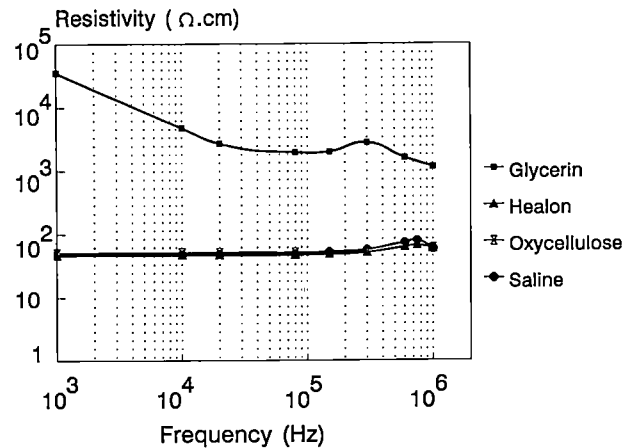


Fig 2. Resistivity as function of frequency at 22°C of impressed sinusoidal current.

of the Nucleus array in container A when it was filled with saline, undiluted hyaluronic acid, or undiluted oxycellulose was a modest 2.5 k Ω at 37°C and 10 kHz and almost completely resistive. Undiluted glycerin displayed a high-pass character with an impedance of 510 k Ω at 10 kHz that dropped to 266 k Ω at 100 kHz. A film of glycerin applied to the electrode array did not affect the impedance across the stimulated electrodes.

The interfacial polarization caused by a film of glycerin did not significantly affect the far-field current spread measured in a tank filled with saline, not even when the common ground stimulation mode was used.

DISCUSSION

In the undiluted form, glycerin was the only lubricant in which the impedance deviated significantly from that of saline. In the frequency range relevant for stimulation of the cochlear nerve, undiluted glycerin displayed a high-pass character. When immersed in undiluted glycerin, the maximum current level for the Nucleus implanted current source would be about 40 μA at 10 kHz, instead of the design specification of 1,500 μA (given the maximum compliance voltage of 20 V peak to peak).

The substances were also applied as a thin film. Only glycerin had an effect on the impedance, including a 180° phase shift. However, a film of glycerin did not affect the impedance between the stimulated electrodes or the far-field current spread measured with distant sensing electrodes. We conclude that a film of glycerin around the electrode array resulted in a polarization layer affecting the current flow along the surface of the electrode array without a substantial effect on the overall volume conduction for the driving current. Far-field effects were not observed even in the common ground stimulation mode, in

which the spatial current distribution is most likely to be affected by impedance shifts.⁹

In sum, none of these lubricants are expected to

alter the current flow toward the excitable elements. They do not seem to affect intraoperative electrically elicited stapedius reflex or auditory brain stem response measurements.

ACKNOWLEDGMENTS — The authors thank Ernst von Wallenberg for his comments on the manuscript. Cochlear UK is acknowledged for providing the electrode array used in this study.

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INTERNATIONAL PEDIATRIC OTOLARYNGOLOGY ENDOSCOPY DAYS

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MENINGIOMA OF THE INTERNAL AUDITORY CANAL

ANTHONY G. ZEITOUNI, MD

MONTREAL, CANADA

DAVID ZAGZAG, MD

NEW YORK, NEW YORK

NOEL L. COHEN, MD

NEW YORK, NEW YORK

Meningiomas are the second most common tumor to involve the cerebellopontine angle (CPA), but controversy exists as to whether they can arise within the internal auditory canal (IAC) or whether involvement of the IAC occurs secondarily by extension from the CPA. This paper reports on a patient with an enhancing IAC meningioma that then grew and on subsequent scans was found to involve the CPA. This case demonstrates that these tumors can arise within the IAC and can grow out to involve the CPA. These findings are discussed within the context of meningioma tumor genetics and the histologic evidence for precursor cells in the IAC. The radiologic findings useful in distinguishing an acoustic neuroma from a meningioma are reviewed in the light of this case. While an enhancing mass projecting into the IAC is most often an acoustic neuroma, this radiologic finding is not pathognomonic.

KEY WORDS — acoustic neuroma, internal auditory canal, meningioma.

INTRODUCTION

Meningiomas are the most common intracranial tumors, and are the second most common tumor to involve the cerebellopontine angle (CPA).^{1,2} They have never been demonstrated to grow from the internal auditory canal (IAC) and to subsequently involve the CPA. In the era before magnetic resonance imaging (MRI), meningiomas were most often discovered when they had grown quite large; thus, their site of origin was uncertain. It was thus unclear whether a meningioma could originate from within the IAC and grow out into the CPA or whether they always originated in the CPA and involved the IAC

secondarily.

With the introduction of MRI with gadolinium, tumors as small as 2 mm could be detected in the IAC. The vast majority of these tumors turn out to be acoustic neuromas. The objective of this paper is to demonstrate that meningiomas should be considered and included in the differential diagnosis of a purely intracanalicular tumor.

CASE REPORT

A 60-year-old woman presented with a 1-month history of aural fullness. She denied hearing loss, tinnitus, or vertigo, but recalled a 3-day episode of

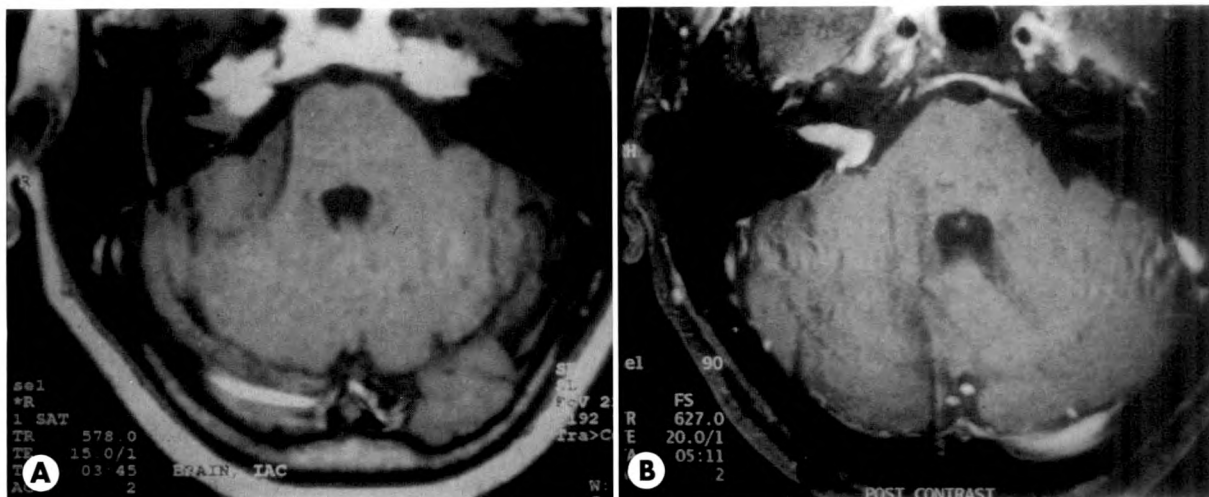


Fig 1. Magnetic resonance imaging with gadolinium. A) Enhancing mass in internal auditory canal, just reaching porus acusticus. B) Growth of enhancing mass over period of 1 year.

From the Department of Otolaryngology, New York University School of Medicine–Bellevue Hospital Center (Zeitouni, Cohen), and the Department of Pathology, Division of Neuropathology, New York University School of Medicine (Zagzag), New York, New York. Dr Zeitouni is presently in the Department of Otolaryngology, Royal Victoria Hospital, McGill University, Montreal, Canada.

CORRESPONDENCE — Noel L. Cohen, MD, Dept of Otolaryngology, New York University Medical Center, 550 First Ave, New York, NY 10016.

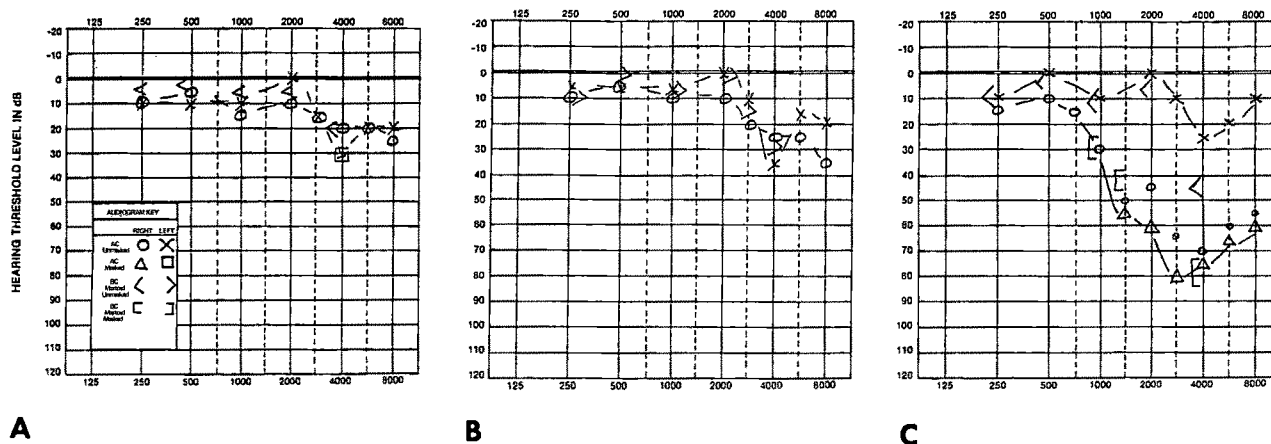


Fig 2. Audiograms demonstrating progression in hearing loss over period of 1 year. A) Mild, asymmetric sensorineural loss at time of diagnosis. Speech discrimination in right ear 100%. B) Increased loss at 6 months. Speech discrimination in right ear 96%. C) Large deterioration in pure tone thresholds 1 year after diagnosis. Speech discrimination in right ear 20%.

disequilibrium 6 months previously. An audiologic workup was ordered and revealed normal, symmetric pure tone thresholds. Discrimination was 100% bilaterally. Acoustic reflexes were absent ipsilaterally and present contralaterally with the probe in the right ear. Auditory evoked potentials demonstrated extremely poor waveform morphology on the right with an unidentifiable wave V. The left ear was normal.

Her symptoms persisted and an MRI scan was ordered that revealed an enhancing lesion in the IAC (Fig 1A) consistent with an acoustic neuroma. Its convex medial surface just reached the porus acusticus. Given her age and excellent hearing (Fig 2A), it was elected to observe the tumor and repeat the MRI every 6 months.

One year after diagnosis, repeat MRI revealed that her tumor had enlarged to fill the IAC and grown 2 to 3 mm into the CPA (Fig 1B). Pure tone testing revealed an asymmetric hearing loss, with a drop in discrimination to 20% in the involved ear (Fig 2C). It was elected to remove the tumor via the translabyrinthine route. At surgery, the tumor was found to be filling the IAC and projecting just outside the porus. The lateral end of the tumor was carefully dissected to ensure complete removal. Separation from the seventh nerve proceeded uneventfully, the eighth nerve was cut, and the tumor was completely excised. Postoperatively, she did well, with normal facial motion and only mild imbalance.

Pathology revealed a meningioma. The tumor had moderate cellularity, there were several meningeal whorls, and tumor cells had nuclear pseudoinclusions (meningotheelial inclusions; Fig 3A). There was no necrosis, and mitotic figures were absent. Immunohistochemical staining revealed the tumor to be vimentin-positive, epithelial membrane antigen-positive, and S-100 protein-negative (Fig 3B,C).

DISCUSSION

The case presented here illustrates that meningiomas can arise within the IAC and can subsequently grow to involve the CPA. Before MRI, meningiomas were detected when quite large, and the site of origin of the meningioma was uncertain; thus, when meningiomas were found within the IAC, it was not possible to determine if they arose there primarily, or whether they involved the IAC secondarily by extension from the CPA, as is not uncommon.³ Previous clinical reports of meningiomas involving the IAC were unable to establish this as the site of origin of the tumor. A case presented by Brookler et al⁴ involving the ampulla of the posterior semicircular canal, IAC, and CPA illustrates the difficulty with determination of the origin. While the extent of the mass was ascertained at surgery, the origin of the mass could not conclusively be established. An exception is the report by Langman et al,⁵ who reported on two cases of meningioma involving the IAC. In one of the cases the tumor, detected by polytomography, was exclusively confined to the IAC. The mass was not followed up over a period of time, so its growth potential could not be evaluated. The second case consisted of a meningioma involving both the CPA and IAC at the time of diagnosis such that its origin could not conclusively be determined.

Clinically, meningiomas most commonly arise during the fourth to sixth decades and have been shown to occur more frequently in women.⁶ They arise most frequently along the dural venous sinuses where arachnoid granulations are found.⁷ Ninety percent of meningiomas are found at supratentorial sites. Of meningiomas affecting the posterior fossa, the CPA accounts for approximately half. That meningiomas may originate within the IAC can be

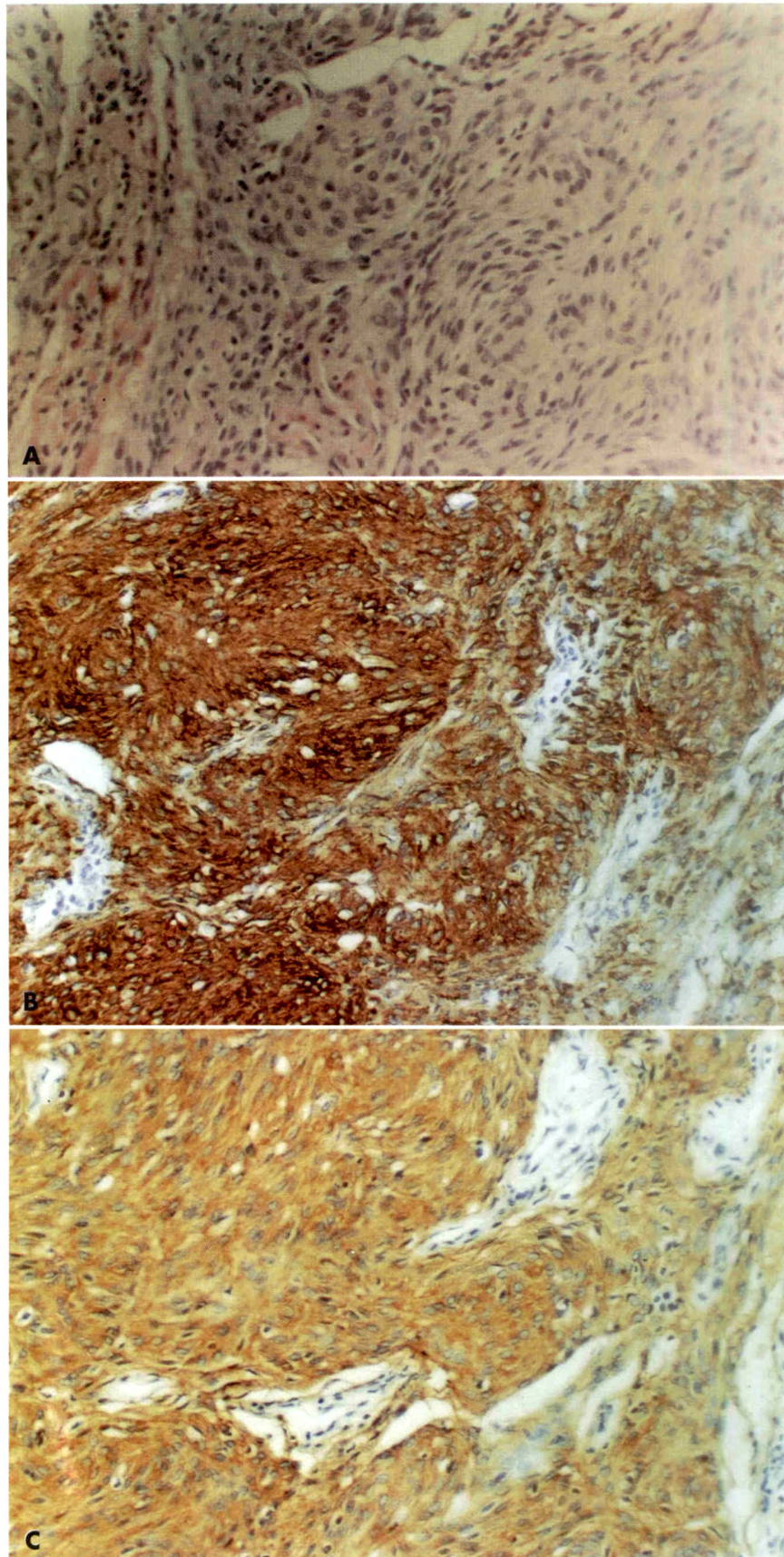


Fig 3. Pathology demonstrating meningioma. A) Hematoxylin and eosin stain. Specimen demonstrated several meningeal whorls, and tumor cells had meningeal inclusions. B) Immunohistochemical staining positive for vimentin. C) Immunohistochemical staining positive for epithelial membrane antigen.

explained by the observation of Nager and Masica,⁸ who reported concentrations of arachnoid cells within the acoustic meatus, as well as at other cranial nerve exit foramina such as the jugular fossa, and at the geniculate ganglion and along the greater and lesser superficial petrosal nerve. The presence of precursor cells in these locations makes it reasonable to expect that meningiomas may occasionally arise in relation to these structures.

Monosomy of chromosome 22 is the most characteristic genetic anomaly.⁹ It appears that a suppressor gene is located on chromosome 22. When both copies of the gene are missing or damaged, unregulated growth can occur, leading to tumor formation. Research by Ruttledge et al¹⁰ supports the contention that lesions in the recently characterized NF2 gene are involved in many spontaneous meningiomas, but other genes on chromosome 22 may also be involved.

Magnetic resonance imaging with gadolinium can detect tumors as small as 2 mm within the IAC. While the vast majority of these tumors turn out to be acoustic neuromas, other diagnoses should be considered. The possibility that an enhancing IAC mass may be a meningioma, as illustrated by this case, has both diagnostic and therapeutic implications. Diagnostic criteria, both clinical and radiologic, have been developed to distinguish acoustic neuromas from meningiomas in the CPA. Clinically, hearing loss has been noted to be less frequent with CPA meningiomas than with similar-size acoustic neuromas.⁷ This case shows that even a small IAC meningioma can cause hearing loss, probably by compromising the cochlear blood supply in the confined space of the IAC as suggested by Langman et al,⁵ who also noted hearing loss associated with their case of IAC meningioma. Radiologically, one criterion to distinguish meningi-

omas from acoustic neuromas has been that meningiomas tend to arise eccentric to the IAC, and do not penetrate the canal.¹¹ As this case illustrates, an origin centered on the IAC, or even a completely intracanalicular lesion, does not rule out a meningioma. When an IAC meningioma grows outside the IAC into the CPA, other radiologic signs, such as a dural tail or calcification on computed tomography, may facilitate the diagnosis.¹¹

The propensity for invasion of the inner ear associated with meningiomas¹² warrants careful identification of the lateral extent of the tumor, both radiologically and at the time of surgery. A translabyrinthine approach is particularly advantageous in this respect, giving an excellent view of the vestibule and lateral end of the IAC. The recurrence rate for posterior fossa meningiomas varies between 0% and 30%. Higher rates are associated with incomplete resection.^{13,14} It has also been suggested that cauterizing adjacent dura, as opposed to excising it with the tumor, may lead to a higher recurrence rate.¹⁵ These reports suggest that the rate of recurrence may be higher than that for acoustic neuromas; however, many of the tumors in these studies had involvement of the skull base or vital structures, hindering their excision and making comparison with acoustic neuroma difficult. Nonetheless, until further observations relating to small CPA and IAC meningiomas are available, a close follow-up of these tumors seems prudent.

CONCLUSION

The case presented here illustrates that meningiomas can arise within the IAC and can subsequently grow to involve the CPA. During growth, these tumors can compromise hearing.

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INTERAMERICAN ASSOCIATION OF PEDIATRIC OTORHINOLARYNGOLOGY

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IMMUNOHISTOCHEMISTRY OF LYMPHOCYTES AND MACROPHAGES IN HUMAN CELLOIDIN-EMBEDDED TEMPORAL BONE SECTIONS WITH ACUTE OTITIS MEDIA

TETSUYA GANBO, MD, DMSC

ISAMU SANDO, MD, DMSC

CAREY D. BALABAN, PHD

CHIAKI SUZUKI, MD, DMSC

MASAHARU SUDO, MD

PITTSBURGH, PENNSYLVANIA

Immunohistochemical analyses were used to investigate the distribution of lymphocytes and macrophages in routine human temporal bone sections obtained from a subject with acute suppurative otitis media. Primary antibodies specific for human CD3 and CD43 (T-lymphocytes), CD20 (B-lymphocytes), CD45 (leukocyte common antigen), and CD68 (macrophages) were used. As a pretreatment, the sections were soaked in antigen retrieval solution (saturated sodium hydroxide-methanol solution in methanol at a ratio of 1:3). A second antigen retrieval procedure (microwave treatment in 1% zinc sulfate) was also employed for identifying CD3-positive cells. Then the avidin-biotin-peroxidase complex technique was performed. Positive reactions to all antibodies but anti-CD68 were observed in the mucosa of the eustachian tube, tympanic cavity, and mastoid air cells. Particularly, cells positive to anti-CD3 or anti-CD43 were making a diffuse invasion upon the lamina propria. CD68-positive cells were scattered only in the effusion of mastoid air cells. These results suggest that the retrospective immunohistochemical study of archival temporal bone sections is a promising approach to investigate the pathogenesis of otitis media.

KEY WORDS — immunohistochemistry, lymphocyte, macrophage, otitis media, temporal bone.

INTRODUCTION

Otitis media is the most common inflammatory disease of the middle ear. Our knowledge of the cellular components of the inflammatory process is limited to studies of aspiration samples of the effusions or biopsy specimens of the mucosa, obtained from the tympanic cavity through the tympanic membrane.¹⁻⁴ Those studies have identified T-lymphocytes, B-lymphocytes, and macrophages. The presence of these inflammatory cells in both the effusions and the mucosa mainly indicates the presence of inflammatory responses in the tympanic cavity. However, there has been no detailed information regarding inflammatory responses in the entire middle ear system, which includes the eustachian tube, tympanic cavity, and mastoid air cells, at the same stage of the disease.

Shi et al⁵ reported that antigen retrieval procedures (soaking in saturated sodium hydroxide [NaOH]-methanol solution) can be used to demonstrate cytoskeletal elements with immunohistochemistry in routine human temporal bone sections (TBSs). Our study modified their original immunohistochemical method to identify differential distributions of T-

lymphocytes, B-lymphocytes, and macrophages in three compartments: the eustachian tube, the tympanic cavity, and the mastoid air cells in archival human TBSs. The initial results demonstrate the feasibility of retrospective immunohistochemical studies of inflammatory processes in otitis media from routine TBSs.

MATERIALS AND METHODS

A 65-year-old man died of renal cell carcinoma. Prior to death, he was neither intubated nor admitted to the intensive care unit. He had a history of otitis media during childhood, and had acute otitis media with purulent effusion at the time of death. The autopsy specimen of human temporal bone was fixed in 10% formalin, decalcified with 5% trichloroacetic acid, and embedded in celloidin block as described previously by Sando et al.⁶ Then, TBSs with 30- μ m thickness were prepared from tissue blocks. The sections were stored in 80% ethanol for 17 years prior to the study.

The TBSs were soaked in distilled water for 60 minutes and then mounted on gelatin-coated slide

From the Elizabeth McCullough Knowles Otopathology Laboratory, Division of Otopathology, Department of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

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CORRESPONDENCE — Tetsuya Ganbo, MD, DMSc, Division of Otopathology, Dept of Otolaryngology, University of Pittsburgh School of Medicine, Eye and Ear Institute Building, Suite 153, 203 Lothrop St, Pittsburgh, PA 15213.

TABLE 1. ZINC SULFATE ANTIGEN RETRIEVAL

Antibody	Microwave Time		
	0 min	5 min	10 min
Anti-CD3	+	++	D
Anti-CD43	+++	++	D
Anti-CD20	+++	++	D
Anti-CD45	+++	+	D
Anti-CD68	+	+	D

Intensity of staining is indicated as follows: + — weak; ++ — moderate; +++ — strongly positive.
D — tissue is damaged.

glasses. A few drops of poly-L-lysine (Sigma, St Louis, Mo) were placed on the slide and the sections were dried at room temperature. The TBSs were then soaked in diluted NaOH-methanol solution for 30 minutes, and rinsed sequentially in methanol (2×15 minutes), 70% methanol (2×15 minutes), and distilled water (2×15 minutes). (The NaOH-methanol solution was prepared as follows. Seventy grams of NaOH was dissolved in 500 mL of methanol. This solution was stored in a dark brown bottle for 1 to 2 weeks to obtain a saturated solution. The saturated NaOH-methanol solution was diluted in methanol at a ratio of 1:3 before use.) After rinsing, some sections were incubated with 1% zinc sulfate in a microwave oven. (Sections were incubated in 1% zinc sulfate solution [pH 5.8; Sigma] in a microwave oven [H2500; Energy Beam Sciences, Agawam, Mass].⁷ The incubation time was varied from 0 to 10 minutes at 60°C.) When the incubation medium was cooled for 15 minutes, they were rinsed twice in distilled water again. If needed, sections were processed by proteolytic treatment with trypsin and rinsed well in phosphate-buffered saline (PBS) twice for 15 minutes. (Proteolytic treatment for enzymatic digestion⁸ was performed with 0.1% trypsin [pH 7.8] containing 0.1% calcium chloride [Sigma]. The temperature was maintained at 37°C for 0 to 15 minutes' incubation in a standard laboratory oven.) Endogenous peroxidase was inactivated by incubation with 3% hydrogen peroxide for 20 minutes, and the sections were rinsed twice in PBS. Nonspecific antibody binding was inhibited by incubation with blocking solution containing 2% nonfat dry milk, 2% normal serum from the species used for the secondary antibody, and 0.1% Triton X-100 (Sigma) for 30 minutes. The sections were incubated with the primary antibodies at 4°C overnight.

A rabbit polyclonal antibody for human CD3 (T-lymphocyte) and mouse monoclonal antibodies specific for human CD43 (T-lymphocyte, clone DF-T1), CD20 (B-lymphocyte, clone L26), CD45 (leukocyte common antigen, clone 2 B11), and CD68 (macrophage, PG-M1) were obtained from DAKO (Carpin-

TABLE 2. PROTEOLYTIC TREATMENT WITH TRYPSIN AND ZINC SULFATE ANTIGEN RETRIEVAL

Antibody	Microwave Time (min)	Trypsin Treatment			
		0 min	5 min	10 min	15 min
Anti-CD3	0	+	+	+D	D
	5	++	+++	+++D	D
Anti-CD68	0	+	+++	+++D	D
	5	+	+	+D	D

Intensity of staining is indicated as follows: + — weak; ++ — moderate; +++ — strongly positive.
D — tissue is digested.

teria, Calif). These primary antibodies were used at a dilution of 1:50 in PBS containing 1% bovine serum albumin (BSA) and 0.1% Triton X-100 (Sigma).

After rinsing in PBS, they were exposed to the biotinylated secondary antibodies (horse anti mouse or goat anti rabbit antibodies, Vector Laboratories Inc, Burlingame, Calif) for 60 minutes at room temperature and then incubated with avidin-biotin-peroxidase complex reagent (Vectastain Elite ABC, Vector Laboratories) for 45 minutes. Biotinylated secondary antibodies were diluted 1:200 in PBS containing 1% BSA.

The color was developed with 0.02% 3,3'-diaminobenzidine • tetrahydrochloride in 4% sodium acetate, plus 0.003% hydrogen peroxide. Sections were dehydrated in graded series of 70%, 95%, and 100% ethanol and then soaked in xylene for 15 minutes twice.

Immunohistochemically studied TBSs with no primary antibodies served as negative controls, and paraffin-embedded tonsil sections were the positive controls.

RESULTS

ESTABLISHMENT OF METHODS

Zinc Sulfate Antigen Retrieval. The effects of this retrieval protocol are summarized in Table 1. Three TBSs were used for each antibody and incubation time. This protocol enhanced CD3-like immunoreactivity, but it reduced CD43-, CD20-, and CD45-like immunoreactivity. The intensity of the CD68-positive reactions did not change. Moreover, all tissue on the slides was damaged when they were incubated by microwave for 10 minutes.

Proteolytic Treatment With Trypsin. The effect of proteolytic treatment with trypsin was tested in regard to improving the positive reaction of CD3 and CD68 in these protocols (Table 2). Sections were exposed to trypsin for 0 to 15 minutes, in combination with zinc sulfate treatment. Three sections were investigated in each group. The CD3-like immunoreaction was most robust with trypsin treatment for 5

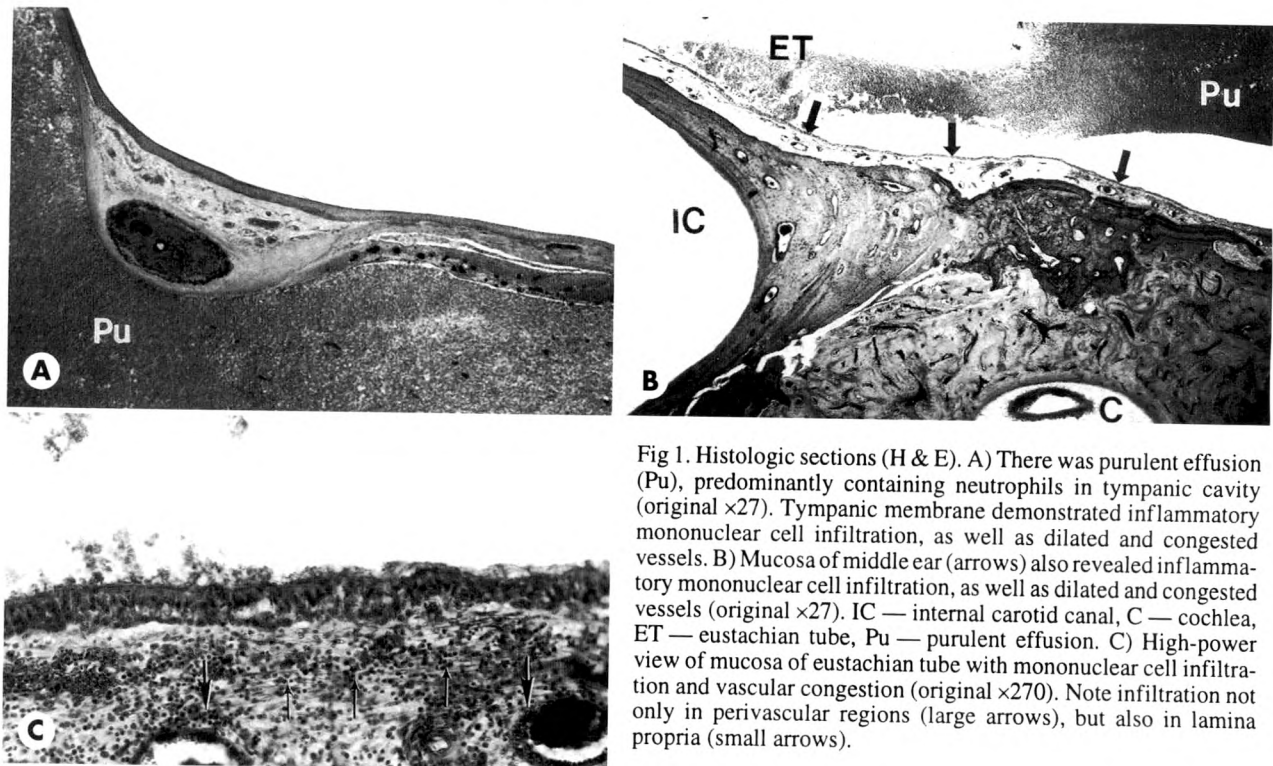


Fig 1. Histologic sections (H & E). A) There was purulent effusion (Pu), predominantly containing neutrophils in tympanic cavity (original $\times 27$). Tympanic membrane demonstrated inflammatory mononuclear cell infiltration, as well as dilated and congested vessels. B) Mucosa of middle ear (arrows) also revealed inflammatory mononuclear cell infiltration, as well as dilated and congested vessels (original $\times 27$). IC — internal carotid canal, C — cochlea, ET — eustachian tube, Pu — purulent effusion. C) High-power view of mucosa of eustachian tube with mononuclear cell infiltration and vascular congestion (original $\times 270$). Note infiltration not only in perivascular regions (large arrows), but also in lamina propria (small arrows).

minutes after a 5-minute microwave incubation with zinc sulfate. Prolongation of trypsin treatment destroyed the tissue. In the case of CD68, the best immunoreaction was produced by a 5-minute trypsin treatment alone.

HISTOPATHOLOGIC FINDINGS

Hematoxylin and eosin staining revealed purulent effusion in the middle ear, which predominantly contained neutrophils (Fig 1A). The tympanic membrane also demonstrated congested vessels and in-

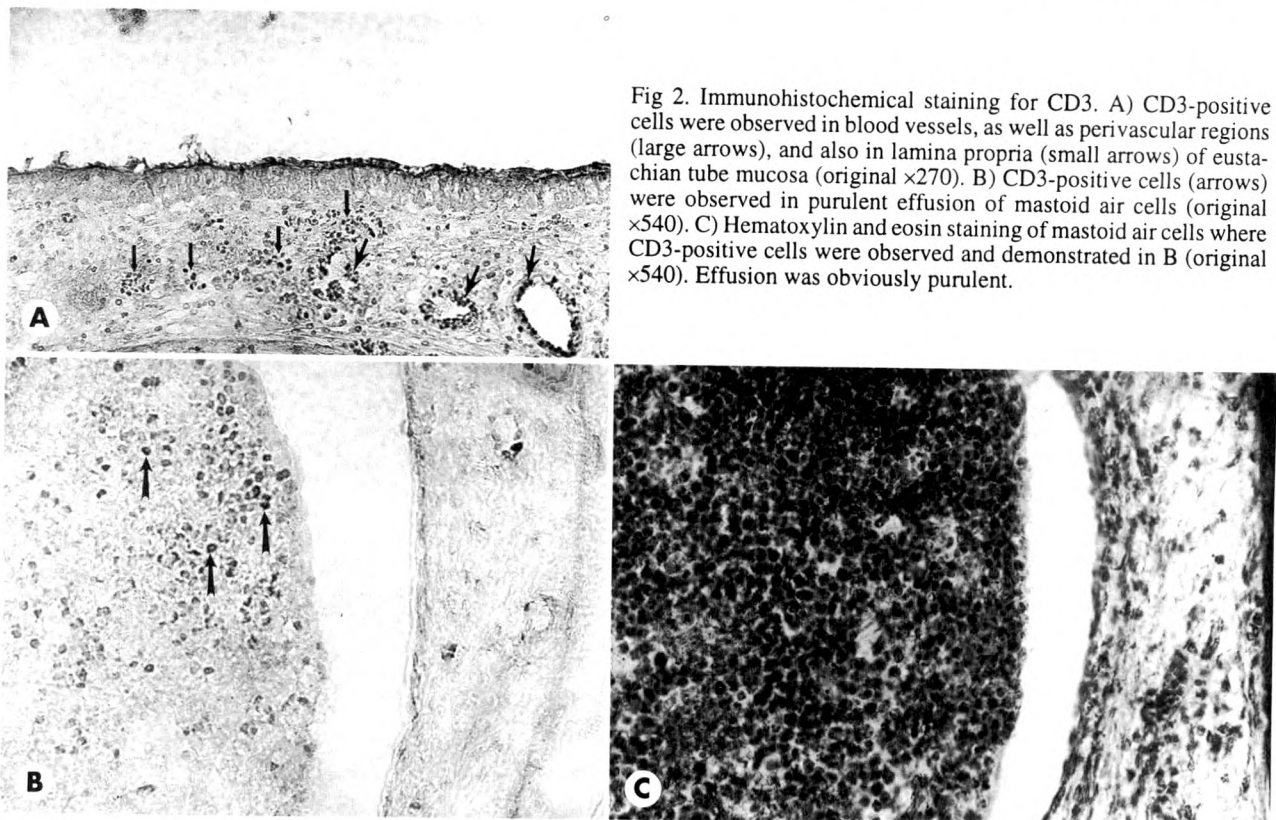
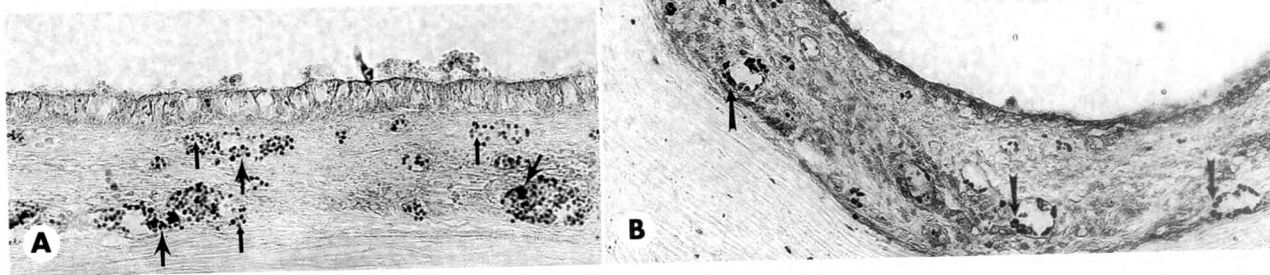


Fig 2. Immunohistochemical staining for CD3. A) CD3-positive cells were observed in blood vessels, as well as perivascular regions (large arrows), and also in lamina propria (small arrows) of eustachian tube mucosa (original $\times 270$). B) CD3-positive cells (arrows) were observed in purulent effusion of mastoid air cells (original $\times 540$). C) Hematoxylin and eosin staining of mastoid air cells where CD3-positive cells were observed and demonstrated in B (original $\times 540$). Effusion was obviously purulent.

Fig 3. Immunohistochemical staining for CD43. A) CD43-positive cells were observed in perivascular regions (small arrows), as well as in blood vessels (large arrows), within mucosa of eustachian tube (original $\times 270$). B) There were many CD43-positive cells (arrows) in mucosa of mastoid air cells (original $\times 135$).



flammatory mononuclear cell infiltration. Furthermore, the mucosa of the tympanic cavity showed dilated and congested blood vessels in the lamina propria with an inflammatory mononuclear cell invasion (Fig 1B). Similar findings were observed in the lamina propria of the eustachian tube (Fig 1C) and mastoid air cells.

The CD3- and CD43-immunopositive cells (presumptive T-lymphocytes) were concentrated in the eustachian tube mucosa (Figs 2A and 3A), the tympanic cavity mucosa, and both the effusion and mucosa within the mastoid air cells (Figs 2B and 3B and Table 3). In the mucosa, they were concentrated in blood vessels and perivascular regions, but they were also distributed diffusely within the lamina propria. Within the purulent mastoid effusion, they tended to be clustered near the outer margin of the cellular mass, near the surface of the inflamed mucosa (Fig 2B,C).

The CD20-immunopositive cells (B-lymphocytes) showed a similar distribution to CD3- and CD43-positive profiles, but were less numerous (Fig 4A,B and Table 3). However, unlike the CD3- and CD43-positive cells, CD20-positive cells were scattered in serous rather than purulent regions of effusion in mastoid air cells (Fig 4C,D).

Cells positive for leukocyte common antigen (CD45) representing both T- and B-lymphocytes were present within the mucosa of the eustachian

tube, tympanic cavity, and mastoid air cells (Fig 5 and Table 3). In addition, they were present in the mastoid effusion. This distribution was also observed for T-lymphocyte (CD3 and CD43) and B-lymphocyte (CD20) markers, but the T- and B-lymphocyte markers together identified more cells in each of these middle ear regions than the CD45 antibody alone (Table 3).

Unlike the lymphocytes, CD68-positive cells representing macrophages were absent in the middle ear mucosa and lumen. These cells appeared to be restricted to the purulent or serous effusion within the mastoid cells (Fig 6 and Table 3).

DISCUSSION

This study demonstrates the feasibility of using celloidin-embedded TBSs for detecting T- and B-lymphocytes and macrophages in a case of acute otitis media.

CD3 is a highly specific marker for T-lymphocytes. The CD3 antibody in this study stains the intracytoplasmic domain of the marker.⁹ On the other hand, CD43 is a human membrane and/or cytoplasmic antigen expressed by T-lymphocyte and myeloid lineage cells. CD43 also shows positive reactions with some B-lymphocytes of B cell leukemia and lymphoma.^{10,11} However, our findings suggest that CD43 expression indicates T-lymphocytes but not B-lymphocytes, because our subject did not have leuke-

TABLE 3. DISTRIBUTION OF CD3, CD43, CD20, CD45, AND CD68

Antibody	Eustachian Tube		Tympanic Cavity		Mastoid Cells	
	Effusion	Mucosa	Effusion	Mucosa	Effusion	Mucosa
Anti-CD3 and anti-CD43 (T-lymphocytes)	—	+++	—	+++	+	+++
Anti-CD20 (B-lymphocytes)	—	++	—	++	±	++
Anti-CD45 (leukocyte common antigen)	—	+	—	+	±	+
Anti-CD68 (macrophages)	—	—	—	—	+	—

Distribution of positive cells is indicated as follows: — negative; ± — rare; + — slight; ++ — moderate; +++ — large.

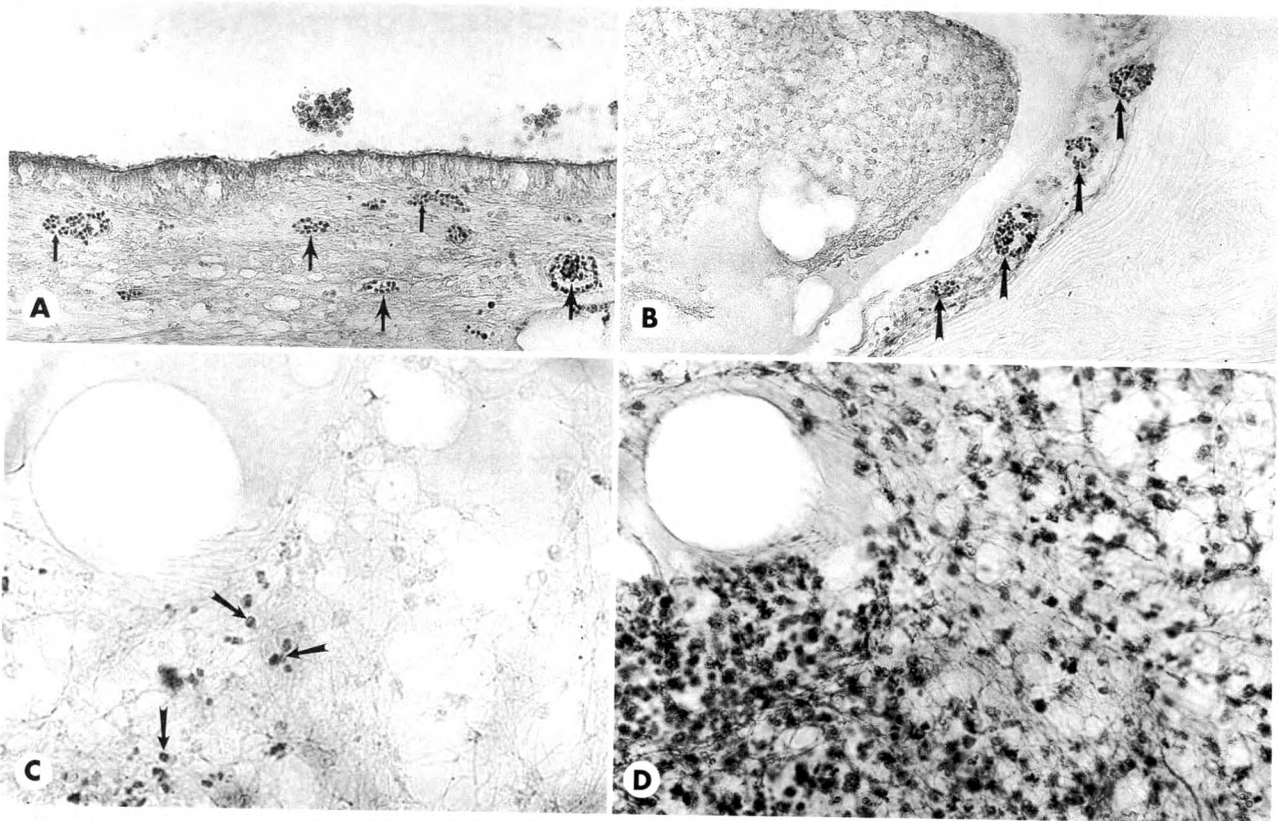


Fig 4. Immunohistochemical staining for CD20. A) CD20-positive cells were mainly observed in blood vessels (large arrows) and perivascular regions (small arrows) within mucosa of eustachian tube (original $\times 270$). B) CD20-positive cells (arrows) were also observed in blood vessels within mucosa of mastoid air cells (original $\times 135$). C) CD20-positive cells (arrows) were rarely detected in serous regions of effusion in mastoid air cells (original $\times 540$). D) Hematoxylin and eosin staining of mastoid air cells with effusion where CD20-positive cells were detected and demonstrated in C (original $\times 540$). Effusion was partially serous, with fibrin deposits.

mia or lymphoma. Consequently, CD43 immunoreactivity revealed the same results as CD3. Positive reactions for CD20 characterize most B-lymphocytes, but not other cell types (eg, T-lymphocytes, monocytes, and granulocytes). Expression of CD20 is abundant in the cytoplasm of B-lymphocytes.^{12,13} Hence, in our temporal bone sections, CD3- or CD43-immunoreactive cells are regarded as T-lymphocytes, and CD20-positive cells as B-lymphocytes.



Fig 5. Immunohistochemical staining for CD45 (original $\times 270$). CD45-positive cells were observed in blood vessels (large arrows) and perivascular regions (small arrows) within mucosa of eustachian tube.

The human leukocyte common antigen (CD45) is found on the surface of T-lymphocytes, B-lymphocytes, granulocytes, and monocytes.¹⁴ However, in our study, there were fewer CD45-positive cells in TBSs than cells positive for CD3, CD43, or CD20. Several factors may account for this result. First,

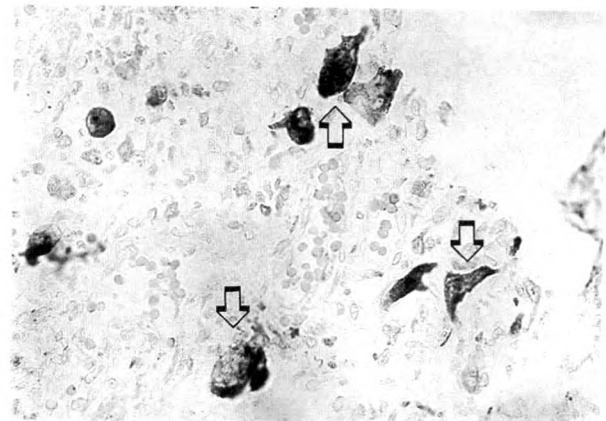


Fig 6. Immunohistochemical staining for CD68 (original $\times 540$). CD68-positive cells (arrows) were distributed in a scattered fashion in effusion of mastoid air cells. Their forms were various; however, most were clearly recognized to have nuclei.

there is a report that the staining of anti-CD45 was not obtained as well with 10% formalin fixation as with periodate-lysine-paraformaldehyde fixation.¹⁵ Second, postmortem proteolysis may have compromised CD45 antigenicity. Third, the celloidin processing and/or prolonged storage in ethanol may be a factor. CD68 is a marker for monocytes and/or macrophages associated with lysosomal granules.¹⁶ Finally, CD68-positive cells could be discriminated from other inflammatory cells such as lymphocytes and neutrophils.

Knowledge as to immunoreactive cells in acute otitis media is based almost exclusively on previous studies that analyzed the fluid from the tympanic cavity.¹⁷⁻¹⁹ However, in addition to the tympanic cavity, it is also important to investigate the distribution of inflammatory cells in the eustachian tube and mastoid air cells to provide a comprehensive view of sites of inflammatory response in otitis media. This study established methodology for addressing this issue in archival human TBSs.

One interesting feature of our case was the differential distribution of inflammatory cells in the effusion. Qvarnberg et al¹⁷ reported that neutrophils were detected predominantly in the aspirated effusions from 87% of untreated cases with acute otitis media. In our immunohistologic study, we could not detect such inflammatory cells as lymphocytes and macrophages in the purulent effusion. Hence, our findings are comparable with those in their report. However, Bernstein et al¹⁸ further suggested that neutrophils and a few T-lymphocytes were present in the purulent effusion at a very early stage of acute otitis media. They also reported that some T-lymphocytes appeared as a form of cell-mediated immunity in the effusion at the next stage, and that B-lymphocytes and macrophages produced an immune response. Moreover, there is a report that macrophages could not be detected in the purulent effusion of acute otitis media within the first few days after onset, but that they increased gradually in number and size with the duration of otitis media as a correlate of resolution of inflammation.¹⁹ In our study, immunoreactive lymphocytes were observed in the mucosa of all three

compartments, while macrophages were detected only in the effusion of the mastoid air cell region.

Further, the majority of immunopositive cells in the purulent effusion were presumptive T-lymphocytes (positive to anti-CD3 or anti-CD43), while B-lymphocytes (CD20-positive cells) were restricted to serous effusion. By contrast, macrophages (CD68-positive cells) were observed in both serous and purulent effusions. The presence of B-lymphocytes and macrophages in some of the mastoid air cells may indicate an advanced stage of suppurative otitis media in this case. However, the significance of differences in the distribution of inflammatory cells within effusions of the eustachian tube, tympanic cavity, and mastoid air cells requires further investigation.

In our case, T-lymphocytes were detected diffusely in the mucosa of the eustachian tube, tympanic cavity, and mastoid air cells, and B-lymphocytes were also observed in blood vessels and perivascular regions in the mucosa in all three compartments. Furthermore, our results may support the theory that the immune response of B-lymphocytes follows the activity of T-lymphocytes. In this case, T- and B-lymphocytes were gathered and accumulated in vessels and perivascular regions within the lamina propria of the mucosa. In our preliminary studies using normal TBSs without otitis media, we detected a small number of T- and B-lymphocytes in the blood vessels with the normal ratio for peripheral blood and a negligible number of lymphocytes within the lamina propria. These findings are consistent with the hypothesis that this case represents acute otitis media in the early active stage.

In conclusion, using antigen retrieval immunohistochemistry, we could detect and differentiate three kinds of inflammatory cells representing T- and B-lymphocytes and macrophages in routine human TBSs in a case of acute otitis media. Furthermore, we observed their differential distribution in the eustachian tube, tympanic cavity, and mastoid air cells. These findings could help us in understanding the roles of immunoreactive inflammatory cells in the pathogenesis of acute otitis media.

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LOCALIZATION OF TRANSFORMING GROWTH FACTOR- β -EXPRESSING CELLS AND COMPARISON WITH MAJOR EXTRACELLULAR COMPONENTS IN AURAL CHOLESTEATOMA

STEPHAN LANG, MD

VOLKER SCHILLING, MD

BRIGITTE MACK

BARBARA WOLLENBERG, MD

ANDREAS NERLICH, MD

MUNICH, GERMANY

Transforming growth factor- β (TGF- β) plays an important role in the regulation of extracellular matrix (ECM) deposition by stimulating the synthesis of individual matrix proteins like tenascin and fibronectin. Cholesteatoma shows significant changes in the ECM, supporting the view of a disturbed cell-matrix interaction. The purpose of our present study was to evaluate the distribution of TGF- β in comparison to the deposition of tenascin, fibronectin, and collagen as major components of the ECM in cholesteatoma ($n = 12$) by means of histochemistry and immunohistochemistry. We found TGF- β in lymphocytes and fibrohistiocytes in the stroma of 7 cholesteatomas. In corresponding sections, a marked expression of tenascin and fibronectin was seen manifesting as a continuous band along the epidermal-stromal junction, extending to the deeper stroma. In addition, in those cases of TGF- β expression, beginning collagen fibril formation was seen in adjacent deeper stroma layers, indicating beginning stromal fibrosis. These results suggest that TGF- β may be involved in the stimulation of the synthesis of tenascin, fibronectin, and collagen. Furthermore, the enhanced expression of tenascin and fibronectin provides evidence for a deregulated cell-matrix interaction in cholesteatoma associated with the enhanced proliferative process of cholesteatoma formation.

KEY WORDS — cholesteatoma, TGF- β , transforming growth factor- β .

INTRODUCTION

The extracellular matrix (ECM), particularly the basement membrane (BM), and thus the cell-matrix interaction are involved in the control of cell morphogenesis, differentiation, and migration.^{1,2} Pathologic ECM formation plays a major role in a variety of metabolic, immunologic, and neoplastic diseases leading to uncontrolled cell growth.^{3,4} Middle ear cholesteatoma is characterized by pathologic cell proliferation resulting in the accumulation of keratin debris, destruction of the surrounding bony structures, and invasion of the inner ear or intracranial cavity.⁵

Recently, we demonstrated significant changes in the composition of the ECM in cholesteatoma, showing a fragmented BM and altered integrin expression as a possible explanation for the proliferative character of that lesion.^{6,7} Much attention has been focused in recent years on the regulation of cell-matrix interaction. One of the most potent regulators for ECM formation known to date is transforming growth factor- β (TGF- β).⁸ We decided to immunohistochemically investigate the distribution of TGF- β in cholesteatoma tissue in comparison to external auditory meatal skin. The results were correlated with the staining patterns of tenascin, fibronectin, and colla-

gen. Since it is known that the synthesis of these ECM components is strongly stimulated by TGF- β , we were able to localize the main TGF- β -producing cells, which can be assumed to play an important role in the pathogenesis of cholesteatoma-associated stroma changes.

MATERIAL AND METHODS

Sampling of Tissue. A total of 12 patients with cholesteatomas was studied. Biopsy specimens of external auditory meatal skin from the edge of the tympanomeatal flap and cholesteatoma, resected en bloc to avoid iatrogenic tissue alteration, were collected during middle ear surgery. After surgical removal, the tissue was snap frozen in liquid nitrogen and stored at -20°C for further use. Informed consent had been given by all patients.

Immunostaining. Polyclonal antibodies against the ECM component fibronectin (anti-fibronectin, Dako, Hamburg, Germany) and monoclonal mouse antibodies against tenascin (Dako) and pan-TGF- β (Genzyme, Cambridge, Mass, recognizing the three isoforms TGF- β 1, - β 2, - β 3) were applied. Frozen sections (4 μm thick) were mounted onto poly-L-lysine-coated slides. Following fixation with acetone, the

From the Departments of Otorhinolaryngology (Lang, Schilling, Wollenberg, Mack) and Pathology (Nerlich), Ludwig-Maximilians-University of Munich, Munich, Germany.

CORRESPONDENCE — Stephan Lang, MD, Grosshadern Medical Center, Dept of Otorhinolaryngology, Marchioninstr 15, 81377 Munich, Germany.

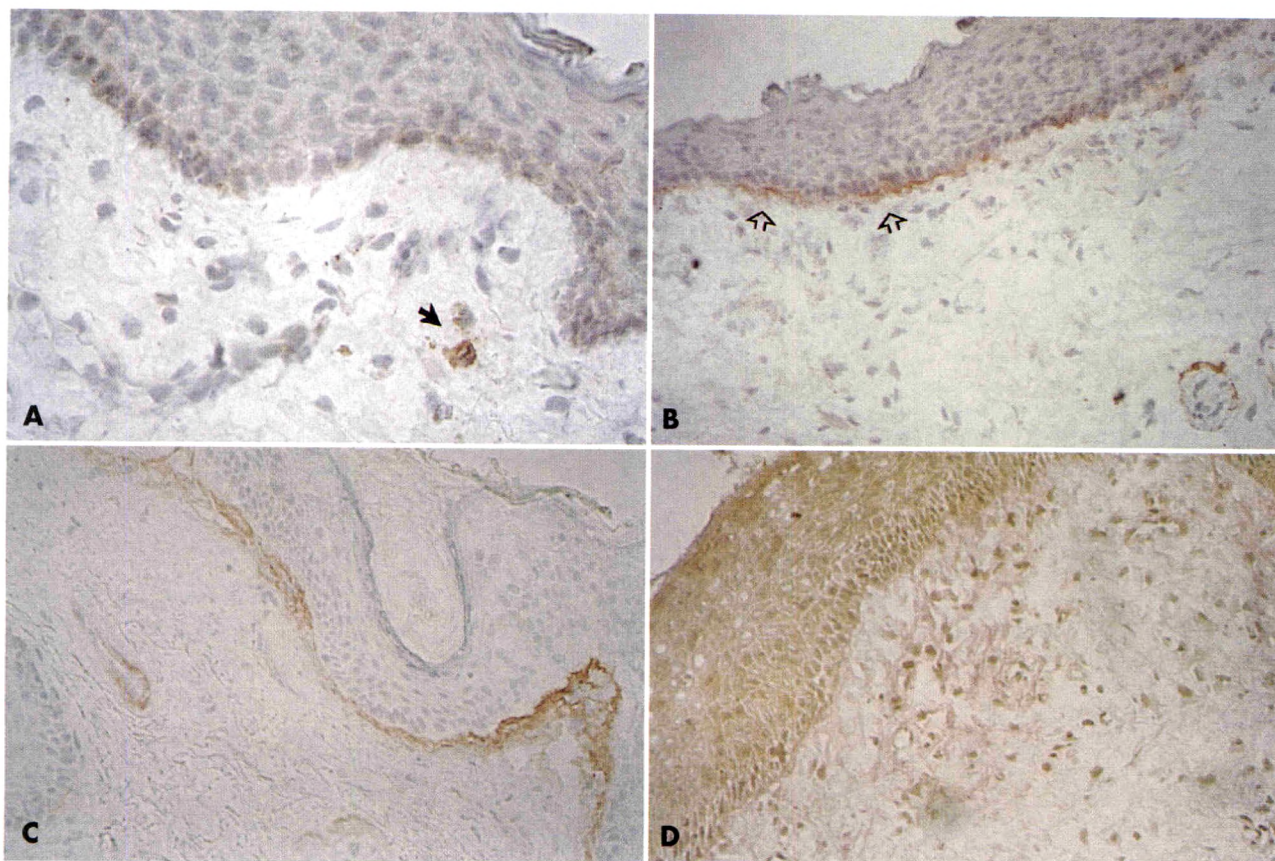


Fig 1. External auditory meatal skin. A) Sparse distribution of transforming growth factor- β (TGF- β) in some lymphocytes and fibrohistiocytes (arrow) of stromal compartment (anti-TGF- β -avidin-biotin-peroxidase complex [ABC-PO], original $\times 365$). B) Moderate expression of tenascin (arrows) restricted to upper dermis (anti-tenascin-ABC-PO, original $\times 228$). C) Distribution of fibronectin with slight staining along epidermal-dermal junction (anti-fibronectin-ABC-PO, original $\times 228$). D) Homogeneous staining of collagenous matrix in dermal stroma (elastica-van Gieson staining, original $\times 228$).

endogenous peroxidase activity was suppressed by treating sections with 0.3% hydrogen peroxidase in phosphate-buffered saline followed by incubation with the primary antibodies against TGF- β , tenascin, and fibronectin. After several washing steps, the sections were incubated with a biotinylated rabbit anti mouse IgG secondary antibody coupled with the avidin-biotin-peroxidase complex (Vectastain, Burlingame, Calif). The respective antigens were visualized by means of the peroxidase reaction with 0.01% 3-amino-9-ethylcarbazole as chromogen (Sigma, St Louis, Mo). After counterstaining with Mayer's hemalum, slides were coverslipped with Kaiser's glycerol gelatin (Merck, Darmstadt, Germany) and examined by light microscopy. In addition, negative control sections were prepared by substitution for the specific antibodies of a rabbit nonimmune serum. Positivity of staining pattern was, in each batch, controlled by the typical staining pattern in lymph follicles of the tonsils (TGF- β), wound healing samples (tenascin), and normal abdominal skin (fibronectin) collected during autopsy.

Histochemical Staining. For the evaluation of the

overall collagen changes, we subjected further frozen sections to van Gieson staining, which selectively stains the collagenous matrix. Appropriate sections were stained as routinely performed.

RESULTS

In external auditory meatal skin, TGF- β was detected very occasionally in a few lymphocytes and fibrohistiocytes of the stroma compartment, while the epithelium showed no significant positive reaction (Fig 1A). In corresponding sections, tenascin reactivity ranged between a patchy and a continuous distribution in the upper papillary dermis adjacent to the BM (Fig 1B). Fibronectin showed a reaction similar to that of tenascin, revealing an almost continuous staining pattern in the BM region (Fig 1C). For both ECM components, the reticular dermis and the epithelial layer showed no positive reaction. With the van Gieson stain, the dermal stroma showed a homogeneous staining of the connective tissue layer (Fig 1D).

In contrast, cholesteatoma tissue showed a strongly enhanced expression of TGF- β in lymphocytes and

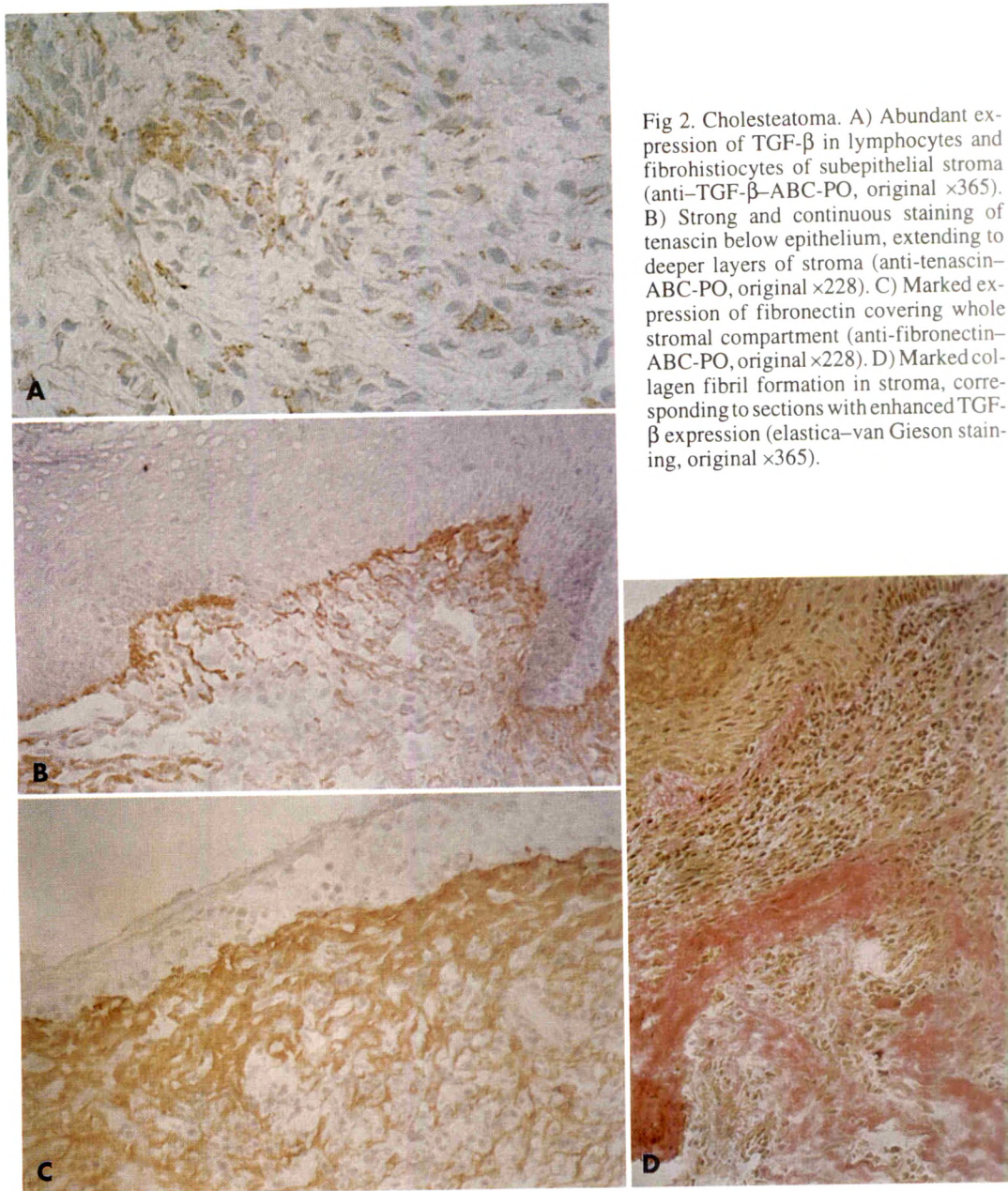


Fig 2. Cholesteatoma. A) Abundant expression of TGF- β in lymphocytes and fibrohistiocytes of subepithelial stroma (anti-TGF- β -ABC-PO, original $\times 365$). B) Strong and continuous staining of tenascin below epithelium, extending to deeper layers of stroma (anti-tenascin-ABC-PO, original $\times 228$). C) Marked expression of fibronectin covering whole stromal compartment (anti-fibronectin-ABC-PO, original $\times 228$). D) Marked collagen fibril formation in stroma, corresponding to sections with enhanced TGF- β expression (elastica-van Gieson staining, original $\times 365$).

fibrohistiocytes of the stroma compartment in 7 of 12 patients, particularly in areas with an inflammatory cellular infiltrate (Fig 2A). In corresponding sections, a significant expression of tenascin and fibronectin was seen as a continuous band, not only restricted to the BM region, but also extending to the deeper stroma (Fig 2B,C). In addition, in those cases of TGF- β expression, beginning collagen fibril formation was seen in deeper layers of the stroma, indicating beginning stromal fibrosis (Fig 2D). In specimens with sparse TGF- β expression (5 of 12), a less pronounced reactivity for tenascin and fibronectin was detected.

DISCUSSION

In recent years, much attention has been focused

on the biology of TGF- β as a potent regulator of cell proliferation. To date, five TGF- β isotypes have been recognized.⁹⁻¹¹ The human TGF- β family consists of the 25 kd polypeptides TGF- β 1, - β 2, - β 3, which are usually secreted as part of a larger, biologically inactive complex showing 70% to 80% homology at the amino acid level.¹² Once activated, TGF- β is involved in the regulation of a variety of processes that depend on the nature of the target cell and the physiological condition, including wound repair, angiogenesis, immunosuppression, control of tumor invasion and metastasis, and cell proliferation.¹²⁻¹⁴ A major activity of TGF- β is the ability to stimulate the synthesis and deposition of ECM proteins like tenascin, fibronectin, and collagen.^{15,16} Moreover, TGF- β blocks matrix degradation by decreasing the syn-

thesis of ECM-degrading proteases.^{12,17,18} Another effect of TGF- β on ECM formation is the stimulation of integrin expression accompanied by augmented cell binding to ECM components.¹⁹ Thus, TGF- β is an important modulator of cell-matrix interaction.

In previous studies, we have reported on a fragmented BM with only minor amounts of laminin and heparan sulfate proteoglycan in aural cholesteatoma indicating a disturbed cell-matrix interaction associated with the proliferative process of cholesteatoma formation.^{6,7} In the present paper, we extended our work on the distribution of TGF- β in cholesteatoma in correlation to the expression of other important ECM components, namely, tenascin, fibronectin, and collagen, which are known to be induced by TGF- β . Tenascin as a modulator of epidermal-mesenchymal interactions and cell proliferation shows a limited distribution in adult connective tissue and is specifically increased in response to epidermal activation.^{20,21} In proliferative skin diseases, an increase of the tenascin expression was found at the epidermal-dermal junction of the dermis, extending to the deeper layers.²² Like tenascin, fibronectin is also found in the dermal compartment adjacent to the BM and is thought to be an important modulator of cell-matrix interaction.² The quantitatively most important ECM component is collagen. For the visualization of collagen fibers as an indicator of stromal fibrosis, we used histochemical van Gieson staining in the present study.

In external auditory meatal skin, a sparse distribution of TGF- β in only some lymphocytes and fibrohistiocytes of the stroma was found, while the staining of tenascin and fibronectin revealed a moderate reaction along the epidermal-dermal junction adjacent to the BM. The low level of TGF- β expression thus correlates with a sparse tenascin and fibronectin content. In the van Gieson stains, the collagenous matrix appeared regularly formed. In contrast, cholesteatoma showed a markedly enhanced expression of TGF- β in stromal lymphocytes and fibrohistiocytes in 7 of 12 patients. In corresponding sections, an abundant and continuous reactivity for tenascin and fibronectin was found in the stromal compartment. Strikingly, the expression of the above-mentioned components was strongest in areas with an infiltra-

tion of inflammatory cells. This is also in accordance with the observation that TGF- β is a potent chemotactic factor leading to the accumulation of inflammatory cells.^{23,24} Released early by platelets in an inflammatory response and subsequently by the inflammatory cells themselves, TGF- β stimulates the synthesis of tenascin and fibronectin.^{25,26} This could be observed in this present study in the subepidermal stroma compartment of cholesteatoma lesions, in which there was a marked expression of these two ECM components.

Matrix induction by TGF- β in cholesteatoma also implicates the stimulation of collagen expression. In cases of TGF- β expression, collagen fibril formation could be demonstrated in deeper layers of the stroma, indicating beginning fibrosis. This would represent an early phase in cholesteatoma development, while the remaining cases ($n = 5$), with only a sparse reactivity for TGF- β , demonstrate a late phase, in which matrix formation or fibrosis was already completed.

Transforming growth factor- β also triggers transcriptional and posttranscriptional regulation of the 92 kd and the 72 kd gelatinase-type IV collagenase, making the enzymatic digestion of ECM²⁷ a possible explanation for the fragmented BM observed in cholesteatoma.⁶ Besides the alteration of the BM, the overexpression of tenascin and fibronectin in cholesteatoma also provides evidence for a deregulated cell-matrix interaction, representing an explanation for the hyperproliferative character of that disease.

In summary, the present work provides evidence that the overexpression of TGF- β in cholesteatoma may be associated with the stimulation of tenascin and fibronectin synthesis, which can be assumed to result in subsequent collagen synthesis. The abundant distribution of these matrix components reveals a deregulated cell-matrix interaction associated with increased cell proliferation in cholesteatoma. The different results of collagen staining may be explained by different stages of cholesteatoma formation: an early phase with beginning stromal fibrosis corresponding to enhanced TGF- β expression, and a late phase characterized by completed matrix formation.

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CLINICOPATHOLOGIC STUDY OF LEPTOMENINGEAL CARCINOMATOSIS INVOLVING THE TEMPORAL BONE

SHUN-ICHI IMAMURA, MD

MAYUMI IMAMURA, MD

IZURU NOZAWA, MD

YOSHIHIKO MURAKAMI, MD

YAMANASHI, JAPAN

The temporal bone pathology of a 71-year-old man with bilateral sensorineural hearing loss and facial paralysis caused by diffuse metastatic leptomeningeal carcinomatosis is described. The origin of this malignant disease was an extremely rare entity, a transitional cell carcinoma of the renal pelvis. Histopathologic study of the temporal bone demonstrated that tumor cells filled the internal auditory meatus, infiltrated into the Rosenthal's canals, and reached the scala tympani of the basal turn of the bilateral cochleas. The vestibulocochlear nerve and facial nerve trunks in the internal auditory meatus had been destroyed by the bilateral tumor invasion. Case reports of temporal bone metastases of leptomeningeal carcinomatosis published since 1965 were reviewed. In leptomeningeal carcinomatosis, it is suggested that tumor cells infiltrate the internal auditory meatus of both ears simultaneously from the cerebrospinal fluid, involving the seventh and eighth nerve trunks, and then cause bilateral sensorineural hearing loss and facial paralysis.

KEY WORDS — bilateral sudden sensorineural hearing loss, facial paralysis, temporal bone histopathology, temporal bone metastasis.

INTRODUCTION

Metastases from solid tumors in the form of diffuse or multifocal seeding to the leptomeninges (leptomeningeal carcinomatosis, leptomeningeal metastases, carcinomatous meningitis) are an important group of neurologic complications of systemic cancer. Leptomeningeal carcinomatosis patients usually have various cerebral, cranial nerve, and spinal root symptoms and signs. Otologic symptoms, including hearing loss, tinnitus, vertigo, and facial weakness, are sometimes seen among the various cranial nerve involvements. However, it is not often that only otologic symptoms are present as the initial clinical complaints. The number of publications on histopathologic findings of the temporal bone in leptomeningeal carcinomatosis has been limited. We present a patient with leptomeningeal carcinomatosis whose chief complaint was bilateral progressive sensorineural hearing loss, followed by bilateral facial nerve paralysis. Finally, this patient had multiple cranial nerve paralyzes and various cerebral and spinal root symptoms. The clinical diagnosis of leptomeningeal carcinomatosis was based on the results of the cerebrospinal fluid (CSF) examination. Both temporal bones were acquired at autopsy for histopathologic examination, and the findings are shown herein. We also review the case reports of leptomeningeal carcinomatosis patients who had otologic symptoms as initial symptoms that include a descrip-

tion of the pathologic findings for the temporal bones. The relationship between the clinical symptoms and the pathologic features of the temporal bones is discussed.

CASE REPORT

A 71-year-old man was admitted to the hospital with the complaint of progressive bilateral hearing loss (Fig 1) of sudden onset on July 21, 1986. Three weeks later (August 14), the audiogram demonstrated

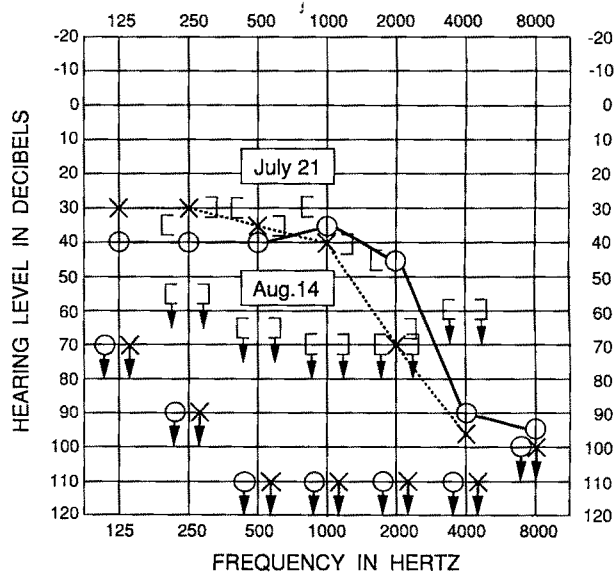


Fig 1. Pure tone audiograms from initial visit (July 21) and 3 weeks later (August 14).

From the Department of Otolaryngology, Yamanashi Medical University, Yamanashi, Japan. This work was supported in part by a Grant in Aid for Scientific Research (No. 05671422) from the Ministry of Education, Science and Culture of Japan.

CORRESPONDENCE — Shun-ichi Imamura, MD, Dept of Otolaryngology, Yamanashi Medical University, 1110, Shimokato, Tamaho-cho, Yamanashi-Ken, 409-38, Japan.

Fig 2. Right ear. Malignant tumor cells (T) have filled internal auditory meatus and destroyed cochlear and vestibular nerve trunks. Tumor cells have reached Rosenthal's canal and scala tympani (arrow-head). There is no tumor cell infiltration or inflammatory reaction in middle ear cavity. Tumor cells have not reached tympanic segment of facial nerve (F).



bilateral total deafness (Fig 1). Six weeks after the onset of hearing loss, he experienced progressive facial weakness, first on the left side and then on the right. The bilateral facial weakness rapidly progressed to complete paralysis. He had had a vertiginous episode 3 months before the onset of hearing loss. However, he only complained of unsteadiness, and had no complaints of vertigo. Although caloric testing of both ears showed no response, spontaneous nystagmus was not observed during the treatment period. These symptoms were then followed by diplopia, dysphagia, and dysarthria, which were suggestive of multiple cranial nerve involvement. Moreover, he then developed various cerebral and meningeal symptoms and signs including headache, nuchal rigidity, absence of deep tendon reflex, and quadriplegia. The examination of the CSF disclosed mark-

edly elevated pressure, protein content, and number of cells. The cytologic examination of the CSF indicated class IV cells, and its results suggested a diagnosis of diffuse metastatic leptomeningeal carcinomatosis. In spite of various clinical studies, the site of origin of the malignant cells that were detected by CSF examination could not be identified while the patient was still alive. No therapeutic measures were effective. His general condition deteriorated and he died on October 8, approximately 3 months after the onset of otologic symptoms.

At autopsy, performed 3 hours after his death, the origin of the malignant tumor was identified as a transitional cell carcinoma of the renal pelvis. The malignant cells had infiltrated diffusely into the leptomeninges and had involved multiple cranial nerves.



Fig 3. Left ear. Findings are almost identical to those of right ear. In addition, mild endolymphatic hydrops is seen in cochlea and saccule. T — tumor cells, F — facial nerve.

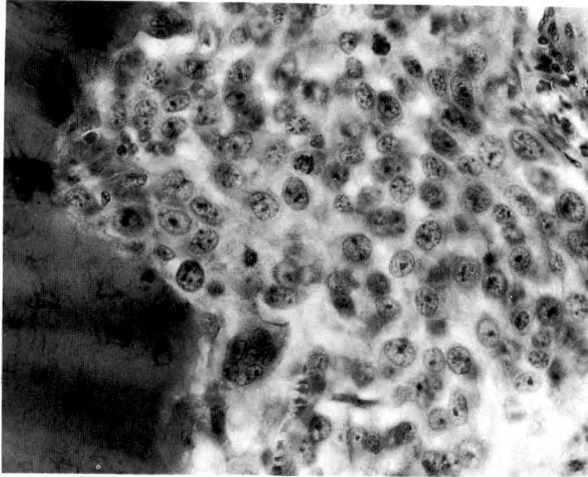


Fig 4. Left ear. Higher-power view of tumor cells in internal auditory meatus. Neoplastic cells have invaded bony wall of internal auditory meatus.

TEMPORAL BONE FINDINGS

Both temporal bones were removed at 3 hours postmortem, fixed in Heidenhain-Susa, and decalcified with 5% trichloroacetic acid. They were then dehydrated and embedded in celloidin. Celloidin blocks were cut at 25 μ m in thickness, and every 10th section was stained with hematoxylin and eosin for light microscopic observation.

The histopathologic findings for the two temporal bones were almost identical except for mild endolymphatic hydrops observed in the left cochlea and saccule (Figs 2 and 3). Bilaterally, the internal auditory meatus was filled with neoplastic cells that had deformed the bony wall of the internal auditory meatus (Fig 4) and replaced the vestibulocochlear and facial nerve trunks. There was diffuse infiltration of neoplastic cells in the modiolus through the cribose area. Moreover, neoplastic cells extended as far as the scala tympani of the basal turn through the osseous spiral lamina and/or the defect of the bony wall of the Rosenthal's canals (Figs 2 and 3, arrowhead). The organ of Corti was partially collapsed, but no tumor cells were found within it, the scala media, or the scala vestibuli. Although mild endolymphatic hydrops was observed in the left ear, there were neither tumor infiltration nor other pathologic findings in the endolymphatic sac and duct. There were no tumor cells in the cochlear aqueduct, and only a few cells were found around the opening to the scala tympani (Fig 5, arrowhead). Tumor cell infiltration was present at the scala tympani of the lower basal turn and on the perilymphatic surface of the round window membrane, but there was no invasion of the middle ear cavity through the membrane (Fig 5, arrow).

The superior vestibular nerve (Fig 6, arrow) and the singular nerve (not shown) had been completely



Fig 5. Right ear. Tumor cells (arrow) have infiltrated scala tympani of lower basal turn and round window membrane, but not invaded middle ear cavity. There are some tumor cells at opening of cochlear aqueduct in scala tympani (arrowhead).

destroyed by tumor invasion. Vestibular ganglion cells could not be identified. However, the neoplastic cells did not extend to the endolymphatic space of the vestibular system (Fig 6).

The facial nerves of both ears had been destroyed by tumor cells in the internal auditory meatus (Fig 7). The tumor cells had invaded the geniculate ganglion, although some facial nerve fibers and geniculate ganglion cells could still be identified. In contrast, there was no tumor cell infiltration in the tympanic or mastoid segments of the facial nerve (F; Figs 2, 3, and 6). The middle ear cavity, tympanic membrane, and external auditory meatus showed no tumor infiltration or inflammatory reaction (Figs 2 and 3).

DISCUSSION

Epidemiology. Clinically, it is known that diffuse



Fig 6. Left ear. Superior vestibular nerve (arrow) shows involvement by tumor invasion. Tumor cells do not extend as far as tympanic segment of facial nerve (F).

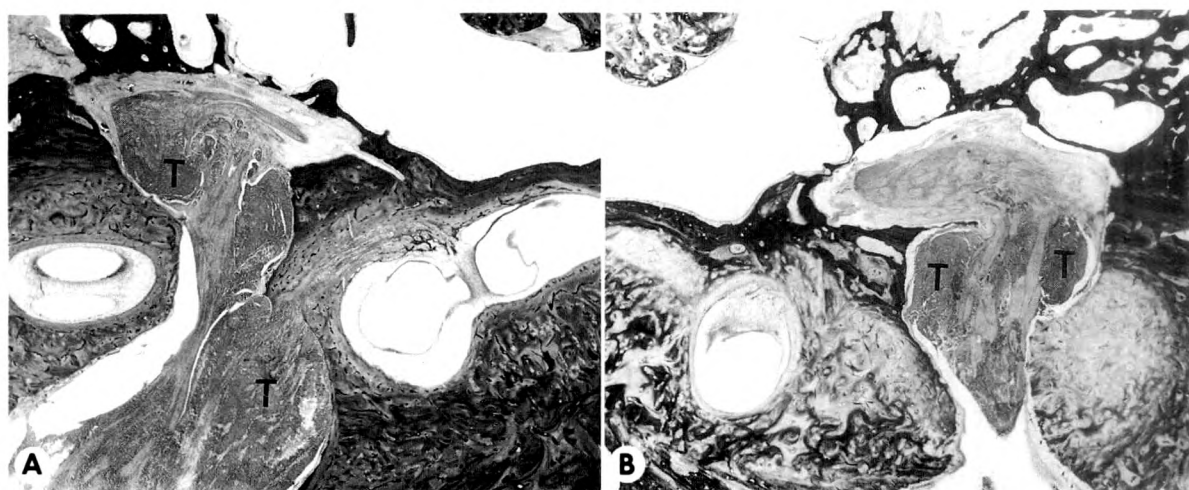


Fig 7. A) Right and B) left ears. Both ears show identical findings. Tumor cells (T) have involved facial nerve fibers and geniculate ganglion. However, some of remaining facial nerve fibers and geniculate ganglion cells can still be identified.

metastatic leptomeningeal carcinomatosis is an important neurologic complication of systemic cancer. The CSF is one of the major pathways by which solid systemic cancer cells reach the leptomeninges and the adjacent structures.¹ Until recently, it was believed that leptomeningeal carcinomatosis was an extremely rare disorder.² It was reported that various cranial nerve palsies usually occurred with cerebral and spinal root symptoms and signs, and that seventh and/or eighth nerve involvement was frequently found

among the symptoms of multiple cranial nerve disorders.^{1,2} However, there are very few reported cases in which the initial clinical presentation included only otologic symptoms without any cerebral or spinal root symptoms. Oshiro and Perlman³ reviewed reports of 11 cases of deafness in carcinomatous meningitis published before 1965.

On the other hand, before the report by Schuknecht et al,⁴ metastatic malignant tumor of the temporal

SUMMARY OF REPORTED CLINICAL AND HISTOLOGIC FINDINGS IN CASES OF METASTASES TO TEMPORAL BONES VIA LEPTOMENINGEAL CARCINOMATOSIS

Authors	Year	Sex	Age	Primary/ Histology	CSF Study	Autopsy	Symptoms	Tumor Invasion Sites
Oshiro and Perlman ³	1965	M	54	Kidney/ adenocarcinoma	—	+	Hearing loss (B), tinnitus, facial palsy	IAM, RC, OS
Hoshino et al ⁶	1972	F	57	Lung/ adenocarcinoma	ND	+	Hearing loss (B), tinnitus, facial palsy	IAM, RC, OS, ST
Nomura et al ⁷	1974	M	26	Stomach/anaplastic adenocarcinoma	ND	+	Hearing loss, vertigo, facial palsy	IAM, RC
Katsarkas and Seemayer ⁸	1976	F	44	Uterine cervix/ epidermoid carcinoma	+	ND	Hearing loss (B), facial palsy, unsteadiness	IAM
Bergstrom et al ⁹	1977	M	52	Bronchus/large cell carcinoma	ND	+	Hearing loss (B), facial palsy, vertigo	IAM, RC
Miyata et al ¹⁰	1985	F	56	Lung/ adenocarcinoma	ND	+	Hearing loss (B), unsteadiness, vertigo	None
Saito et al ¹¹	1985	F	55	Lung/small cell carcinoma	ND	+	Facial palsy, vertigo, hearing loss	IAM
Ohira et al ¹²	1991	M	25	Pancreas/ adenocarcinoma	ND	+	Vertigo, hearing loss (B), facial palsy	IAM, CA
Present study		M	71	Renal pelvis/ transitional cell carcinoma	+	+	Hearing loss (B), tinnitus, facial palsy	IAM, RC, OS, ST

CSF — cerebrospinal fluid, ND — not described, B — bilateral involvement, IAM — internal auditory meatus, RC — Rosenthal's canal, OS — ossaceous spiral lamina, ST — scala tympani, CA — cochlear aqueduct.

bone, especially metastasis via meningeal carcinomatosis, was also thought to be a very rare disorder. Recently there has been an increase in the number of cases of temporal bone metastases.⁵ Most of these cases were due to hematogenous dissemination from an isolated primary tumor and/or direct invasion from an adjacent tumor.^{4,5} We reviewed the case reports^{3,6-12} that included a description of the pathologic findings of temporal bone metastases through leptomeningeal carcinomatosis that have been reported since Oshiro and Perlman's³ report. Each case is summarized in the Table.^{3,6-12} The patients all showed otologic symptoms, including hearing loss and/or tinnitus, facial paralyses, and vertigo as the initial symptoms of leptomeningeal carcinomatosis, and the diagnoses had been based on the presence of malignant cells in the CSF or the detection of tumor invasion to leptomeninges on biopsy or autopsy.

Generally, it is believed that leptomeningeal metastases most frequently originate from breast carcinoma,^{1,2} and it was reported that the most common primary site of temporal bone metastases of malignant tumor is also breast carcinoma.^{4,5} However, in our review, the cases of primary tumor of leptomeningeal carcinomatosis with otologic symptoms included three cases in which the primary tumor was located in the lung, and one each in which it was in the kidney, stomach, uterine cervix, bronchus, and pancreas. There was no case other than our case with transitional cell carcinoma of the renal pelvis as the primary malignant tumor among the cases of leptomeningeal carcinomatosis^{1,2} or among the cases of temporal bone metastasis.^{5,6}

Clinical Findings. Leptomeningeal carcinomatosis is characterized clinically by various neurologic symptoms and signs involving more than one level of the neuraxis.¹ In the terminal stage of the disease, our patient ultimately presented the typical central nervous system dysfunction, including headache, nausea (cerebral symptoms), hearing loss, facial paralyses, diplopia, dysphasia, dysarthria (cranial nerve symptoms), and quadriplegia (spinal symptom). In general, metastatic tumors of the temporal bone cause symptoms that are similar to those of otitic infection such as otalgia, otorrhea, mass in the external auditory meatus, retroauricular swelling, and conductive hearing loss.^{4,5,13} In contrast, our review of the temporal bone metastases through leptomeningeal carcinomatosis did not disclose any middle ear symptoms, and in all cases the hearing loss was exclusively sensorineural in nature. Routine microscopic examinations of the temporal bones from patients who had died of malignant tumors reveals that there is more frequent tumor involvement of bilateral temporal bones than once thought.⁵ Bilateral tumor involvement

is observed even in patients who had no otologic symptoms.⁵ In general, patients with temporal bone metastasis commonly have unilateral symptoms at the early stage, although there is occasionally the exceptionally rare case in which bilateral symptoms are initially seen simultaneously without leptomeningeal metastasis.¹⁴ In contrast, in all the reviewed leptomeningeal carcinomatosis cases and additional cases with no pathologic examination,^{15,16} the otologic symptoms were frequently bilateral, and often showed sudden onset and/or rapid progression. In most of the previously reported cases the initial symptom was hearing loss, and facial paralysis usually developed later, as in our case. This sequence may be due to differences in vulnerability between the facial nerve (motor neuron) and the vestibulocochlear nerve (sensory neuron), as well as in the location of tumor cells in the internal auditory meatus. Episodic vertigo was less frequent than we expected. In our case, clinical examinations, including computed tomography and magnetic resonance imaging performed by the neurosurgery department, did not show the origin of the initial vertigo attack. The vertiginous episode may have been the initial symptom of the tumor invasion to the leptomeninges. However, even in patients with no caloric response, often the only complaint is unsteadiness, sometimes lacking in episodic vertigo or spontaneous nystagmus, exactly as in our case. This pattern could be due to simultaneous and symmetric tumor invasion of bilateral vestibular nerves.

Clinically, the presence of characteristic symptoms such as bilateral progressive hearing loss and facial paralyses strongly suggests temporal bone metastases through leptomeningeal carcinomatosis. However, it is reported that hematogenous metastasis to the temporal bone can in rare cases cause similar otologic symptoms.¹⁴ Therefore, the final diagnosis of leptomeningeal carcinomatosis should be based on the results of CSF examination or histologic examination of leptomeninges by biopsy or at autopsy.

Temporal Bone Histopathology. The histologic study of temporal bone metastasis is being reported with increasing frequency, although reports on leptomeningeal carcinomatosis are still rare. In all of the reviewed cases except one, tumor cells were found in the internal auditory meatus and had destroyed the vestibulocochlear nerve. In some cases, few or no tumor cells were found in the internal auditory meatus, possibly because of the effects of antineoplastic therapy. Neoplastic cells frequently invaded Rosenthal's canal, and in some cases reached the osseous spiral lamina. It is supposed that tumor cells that have invaded the scala tympani may infiltrate and destroy the bony wall of Rosenthal's canal

and/or the osseous spiral lamina. Ohira et al¹² reported a case showing tumor infiltration into the scala tympani and cochlear aqueduct without invasion of Rosenthal's canal. The cochlear aqueduct may be another tumor pathway of access to the scala tympani, although in our case, tumor cells were found only at the opening of the cochlear aqueduct.

In cases of metastasis to the temporal bone via

leptomeningeal carcinomatosis, diagnosis is difficult in a patient who complains of only a few cranial nerve disorders and does not complain of any other symptoms that would be caused by a primary cancer. In cases of bilateral progressive seventh and eighth nerve disorders, it is important to suspect the possibility of leptomeningeal carcinomatosis and to perform repeated CSF examinations even if there are no systemic malignant symptoms.

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EFFECT OF PHOTODYNAMIC THERAPY ON SELECTED LABORATORY VALUES OF PATIENTS WITH NASOPHARYNGEAL CARCINOMA

JIN P. LAI, MD, PHD

ZHENG D. TAO, MD
SU P. ZHAO, MD, PHD

JIAN Y. XIAO, MD
YONG Q. TIAN, MD, PHD

CHANGSHA, PEOPLE'S REPUBLIC OF CHINA

We determined pre- and post-photodynamic therapy (PDT) serum levels of soluble interleukin-2 receptor (SIL-2R) and interleukin-2 (IL-2), as well as activity of natural killer (NK) cells, among 24 patients with either persistent or recurrent nasopharyngeal carcinoma (NPC), using a double-antibody sandwich technique, tritiated thymidine-deoxyribonucleoside incorporation, and iodine 125-uridine-deoxyribonucleoside release techniques, respectively. The results showed that the post-PDT serum level of SIL-2R had significantly declined ($p < .0005$), while that of IL-2 and the NK cell activity had significantly increased ($p < .0005$), compared with pre-PDT values, suggesting an immunoenhancing effect of PDT on NPC patients.

KEY WORDS — interleukin-2, laser photodynamic therapy, natural killer cell, soluble interleukin-2 receptor.

There have been studies indicating that a certain degree of cellular immunologic incompetence can be shown in malignancy-bearing hosts, and one of the important indicators for such immunologic incompetence is a decreased level of serum interleukin-2 (IL-2).^{1,2} Although a few reports have appeared in the recent literature regarding photodynamic therapy (PDT) of nasopharyngeal carcinoma (NPC), one of the most prevalent malignancies in southern China,^{3,4} no report has been found of the impact of PDT on the serum levels of both soluble interleukin-2 receptor (SIL-2R) and IL-2, as well as on the natural killer (NK) cell activity of patients with NPC. In the present study, both pretherapy and posttherapy serum levels of SIL-2R and IL-2, as well as NK cell activity, were assayed in 24 NPC patients treated with PDT in order to observe the impact.

MATERIALS AND METHODS

Of the 24 patients enrolled, 17 had recurrent NPC after irradiation, while 7 had persistent tumors. There were 20 men and 4 women. Their ages ranged from 20 to 68 years, with an average of 48.2 years. All cases were confirmed histopathologically: 23 were poorly differentiated squamous cell carcinomas, and 1 was an undifferentiated carcinoma. Twenty healthy blood donors were used as normal controls, of whom 12 were men and 8 women, with an average age of 32.5 years. Venous blood samples were drawn, and the sera were separated and then stored at -20°C until

assay.

Reagent kits for detection of SIL-2R were provided by the Department of Immunology, Bethune Medical University, ChangChun, People's Republic of China. Tritiated thymidine-deoxyribonucleoside (^3H -TdR) and iodine 125-uridine-deoxyribonucleoside (^{125}I -UdR) were from the Chinese Institute of Nuclear Energy, and PSD007, a new kind of hematoporphyrin derivative, was produced in the 523 Research Laboratory of the Second Military Medical University of the People's Liberation Army, China.

Therapy began with gasification of the lesion visible to the naked eye with a neodymium:yttrium-aluminum-garnet (YAG) laser (0 to 100 W), with the laser probe of a 400- μm -diameter flexible quartz fiber being introduced transorally to the nasopharynx. Optimally accurate laser gasification was made possible under nasopharyngoscopic monitoring. A photosensitizer (PSD007) skin test was then performed prior to PDT. Only those patients with a negative skin test result were given an intravenous bolus of PSD007 (5 mg/kg), followed by the photoirradiation of the already superficially gasified lesion 24 hours later with a pulsed copper vapor laser system (manufactured by ZheJiang University, HangZhou, China). For PDT, we employed the same kind of laser probe and flexible quartz fiber used with the YAG laser, and the probe was introduced into the nasopharynx either transnasally or transorally. The laser illumination lasted for approximately 40 minutes per session;

From the Department of Otolaryngology, Ear, Nose, and Throat Research Laboratory, Hsiang Ya Hospital, Hunan Medical University, Changsha, People's Republic of China. Granted by the Chinese Medical Board.

CORRESPONDENCE — Jin P. Lai, MD, PhD, Dept of Otolaryngology, Hsiang Ya Hospital, Hunan Medical University, Changsha, 410008, People's Republic of China.

TABLE 1. CIRCULATING SERUM LEVELS OF SIL-2R AND IL-2 AND NK CELL ACTIVITY OF NPC PATIENTS AND NORMAL CONTROLS

	SIL-2R		IL-2		NK Cells	
	n	Mean $\pm$ SD (U/mL)	n	Mean $\pm$ SD (U/mL)	n	Mean $\pm$ SD (%)
Controls	20	230.8 $\pm$ 106.7	20	59.9 $\pm$ 24.9	20	29.1 $\pm$ 9.4
Patients	24	779.4 $\pm$ 214.8	24	27.5 $\pm$ 16.4	24	11.8 $\pm$ 4.1
t-test	p < .0005		p < .0005		p < .0005	

SIL-2R — soluble interleukin-2 receptor, IL-2 — interleukin-2, NK — natural killer, NPC — nasopharyngeal carcinoma.

the wavelength and power were 630 nm and 500 mW, respectively. Additional sessions of photoirradiation were given 48 and 72 hours after the infusion of PSD007. All patients were protected from exposure to sunshine both during the therapy and for 1 month subsequently.

The SIL-2R was assayed with a double-antibody sandwich technique⁵; ie, 40-well polybenzenethyl microplates were first coated with monoclonal rabbit anti human SIL-2R serum (5 mg/mL) and left at 4°C overnight. One percent bovine serum albumin was added and left for 2 hours to block the nonspecific reaction. After washing, each well was filled with 100 μ L sample diluent plus either 50- μ L standards of varied dilutions or 50- μ L serum samples and left at 37°C for 2 hours. Once more, 100 μ L monoclonal antibody SIL-2R was added after washing and left at 37°C for 2 hours. The second antibody used was horseradish peroxidase-conjugated donkey anti rabbit IgG serum, which was also brought to reaction at 37°C for another 2 hours. Washing and coloration were done in a routine manner. The reaction was terminated by addition of 50 μ L of 2 mol hydrogen sulfoxide, and the optical density (OD) values were measured at 490 nm. The levels of serum SIL-2R could be obtained from a standard curve plotted with the OD values.

Induction and quantitation of IL-2² was performed as follows. Peripheral blood mononuclear cells (1×10^6 /mL) were incubated with 1% phytohemagglutinin (PHA) in 37°C, 5% carbon dioxide for 48 hours. The supernatants were harvested to detect the IL-2 therein induced by means of the ³H-TdR technique and with cultured T-lymphocyte line-2 (CTIL-2) as

the detector. The counts per minute (cpm) values acquired could be easily converted to the active units of IL-2.

Detection of NK cell activity² was performed as follows. Peripheral blood mononuclear cells were diluted with RPMI 1640 to a concentration of 1×10^7 /mL, and the target cells (k562) were labeled with ¹²⁵I-UdR. A mixture of both effector cells and target cells with an effector/target ratio of 100 was put on 96-well microplates and incubated at 37°C, 5% carbon dioxide for 17 to 18 hours. After measurement of cpm, the NK activity was derived from the following formula.

NK activity =

$$\frac{\text{cpm (sample)} - \text{cpm (natural release)}}{\text{cpm (maximum release)} - \text{cpm (natural release)}} \times 100\%$$

RESULTS

The serum levels of SIL-2R and IL-2, as well as the NK cell activity, for both NPC patients and normal controls are shown in Table 1, from which it is clear that SIL-2R was significantly greater ($p < .0005$) in NPC patients than in normal controls, while the opposite was true for both the other parameters ($p < .0005$).

Both the pre- and post-PDT serum levels of SIL-2R and IL-2, as well as the NK cell activity, of the NPC patients are shown in Table 2, from which it is also clear that as compared with pre-PDT, the post-PDT serum level of SIL-2R was significantly decreased ($p < .0005$), while the levels of IL-2 and the NK cell activity was significantly increased ($p < .0005$).

TABLE 2. PRE- AND POST-PDT CIRCULATING SERUM LEVELS OF SIL-2R AND IL-2 AND NK CELL ACTIVITY OF NPC PATIENTS

	SIL-2R		IL-2		NK Cells	
	n	Mean $\pm$ SD (U/mL)	n	Mean $\pm$ SD (U/mL)	n	Mean $\pm$ SD (%)
Pre-PDT	24	779.4 $\pm$ 214.8	24	27.5 $\pm$ 16.4	24	11.8 $\pm$ 4.1
Post-PDT	24	346.5 $\pm$ 178.3	24	52.8 $\pm$ 29.6	24	26.4 $\pm$ 8.2
t-test	p < .0005		p < .0005		p < .0005	

PDT — photodynamic therapy, SIL-2R — soluble interleukin-2 receptor, IL-2 — interleukin-2, NK — natural killer, NPC — nasopharyngeal carcinoma.

There were significant negative correlations both between the pre-PDT serum levels of SIL-2R and IL-2 ($r = -.576$, $p < .01$) and between the pre-PDT serum level of SIL-2R and the pre-PDT NK cell activity ($r = -.714$, $p < .01$), while the pre-PDT level of IL-2 was significantly positively correlated with that of the NK cell activity ($r = .662$, $p < .001$).

DISCUSSION

As is well known, PDT is based on the selective accumulation of the photosensitive agent in the neoplastic tissue rather than in the normal tissue and, therefore, is able to exert a tumoricidal effect by activating this agent with a light of definite wavelength. Both animal studies and clinical trials have demonstrated the efficacy of PDT in cases of malignancies. The photosensitive agent most widely used so far, both domestically and overseas, is hematoporphyrin derivative (HpD), and the most frequently applied light source has been the laser. What we used in the present study was PSD007, which, as a new-generation photosensitive agent, possesses a much stronger photodynamic effect on NPC than does HpD.⁶

With regard to the effect of PDT on the immunologic function of the organism, Liang et al⁷ reported that while a small dosage (10 mg/kg) of HpD in the absence of light illumination exhibited an immunoenhancing effect on mice, an immunosuppressive effect, on the contrary, could be observed with a rather large dosage (20 mg/kg), and Zhao et al³ did not find any evidence of PDT-induced suppression of lymphocyte blastogenesis. As is well known, cellular immunity plays a major role in body defenses to the development of cancer. One of the important indica-

tors of damaged immune function is the reduction of IL-2.^{1,2} It has been established that IL-2 produced by lymphocytes can promote lymphocyte proliferation, release various lymphokines, intensify T-lymphocyte-induced cytotoxicity, stimulate the proliferation and differentiation of NK cells, induce the production of γ -interferon, and directly or indirectly accelerate the Ig production of B-lymphocytes.⁸ Rey et al² reported a significant reduction of circulating IL-2 level and NK activity in a case of solid tumor, suggesting decreased immune function. IL-2R is a specific molecular protein expressed on the cell membrane of the activated T-lymphocyte, of which those released into the circulating blood have been designated as SIL-2R. Since SIL-2R can compete with IL-2R for the binding site of IL-2, leading to the reduction of the circulating IL-2 level, an abnormally increased level of circulating SIL-2R may suppress the immune function of the organism.⁹ This also may explain why many diseases with an immune dysfunction, such as leukemia, show an increased level of SIL-2R. Until now, no study has been done on whether there is any impact of PDT on the levels of circulating SIL-2R or IL-2 or on NK cell activity. The results of the present study showed a significantly increased level of serum SIL-2R, as well as a significantly decreased level of serum IL-2 and NK cell activity, before PDT. They also showed a significant reduction of the serum SIL-2R level, as well as a significant increase of the serum IL-2 level and NK cell activity, after PDT. They also showed either a significantly positive or significantly negative correlation between SIL-2R and IL-2 and NK cell activity. Therefore, it can be concluded that PDT may have an immunoenhancing effect on the tumor-bearing host.

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EFFECTS OF INDOMETHACIN, DEXAMETHASONE, AND ERYTHROMYCIN ON ENDOTOXIN-INDUCED INTRAEPITHELIAL MUCUS PRODUCTION OF RAT NASAL EPITHELIUM

YUKIMITSU TAKAHASHI, MD

TAKESHI SHIMIZU, MD

YASUO SAKAKURA, MD

TSU, JAPAN

We produced hypertrophic and metaplastic changes of goblet cells in rat nasal respiratory epithelium by the intranasal instillation of endotoxin (ETN). In the present study, we examined in vivo effects of indomethacin (IND), dexamethasone (DEX), and erythromycin (EM) on intraepithelial mucus production using this animal model. Intraperitoneal injection of IND (2 to 4 mg/kg body weight $\times$ 4 days) or DEX (4 to 8 mg/kg body weight $\times$ 4 days) significantly inhibited intraepithelial mucus production induced after 3 days of ETN instillations. Intraperitoneal injection of EM (100 mg/kg body weight $\times$ 8 days), aminobenzylpenicillin (ABPC, 200 mg/kg body weight $\times$ 8 days), and cephalothin (CET, 200 mg/kg body weight $\times$ 8 days) also inhibited intraepithelial mucus production induced after 7 days of ETN instillations. When compared with ABPC and CET, EM had a greater inhibitory effect. These results indicate that ETN-induced intraepithelial mucus production can be inhibited by treatment with the anti-inflammatory drugs IND and DEX. Antibiotics such as EM, ABPC, and CET will also be effective, probably by preventing secondary bacterial infection, and EM has an additional inhibitory effect on intraepithelial mucus production.

KEY WORDS — antibiotics, endotoxin, erythromycin, goblet cell, mucus, nasal epithelium, rat, steroid.

INTRODUCTION

Hypertrophy, hyperplasia, and metaplasia of secretory cells associated with hypersecretion of mucus are the important pathologic changes observed in airway inflammation (rhinitis, sinusitis, and tracheo-bronchitis). Anti-inflammatory drugs and steroids have been used in the treatment of these airway conditions. Experimentally, goblet cell hyperplasia has been produced in the rat trachea by the inhalation of tobacco smoke^{1,2} or by the instillation of human neutrophil products.³ These changes can be inhibited by treatment with anti-inflammatory drugs and steroids.^{1,2,4} For elucidating the mechanisms of intraepithelial mucus production, we have produced hypertrophic and metaplastic changes of goblet cells in rat nasal epithelium induced by the intranasal instillation of endotoxin (ETN).⁵ In the present study, we first examined in vivo effects of indomethacin (IND) and dexamethasone (DEX) on ETN-induced mucus production using this animal model.

Erythromycin (EM) and other antibiotics have also been used in the treatment of airway inflammation. Recently, "low-dose, long-term" EM treatment has been reported to be very effective for patients with diffuse panbronchiolitis.⁶ The usefulness of this treatment has been extended to patients with chronic airway diseases such as chronic bronchitis, bronchiectasis, bronchial asthma, and chronic sinusitis.⁷

However, the mechanism of these clinical improvements is still unclear. It has been suggested that these effects of EM depend on its anti-inflammatory action rather than its antibacterial one. In the present study, the in vivo effect of EM on intraepithelial mucus production was examined with this animal model, compared with that of other antibiotics: aminobenzylpenicillin (ABPC) and cephalothin (CET).

MATERIALS AND METHODS

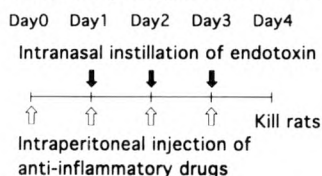
Animals and Nasal Instillations. Male F344 rats (specific pathogen-free, Japan SLC, Inc, Shizuoka, Japan) were used at 8 to 10 weeks of age in this study. The rats were anesthetized with ether, and 0.1 mL of saline containing 0.1 mg of lipopolysaccharides (ETN) from *Escherichia coli* 0111:B4 (Sigma Chemical Co, St Louis, Mo) was instilled into both airways of the nasal cavity (0.05 mg of ETN to each nostril) once a day for 3 or 7 days. The instillate was deposited as a bead of fluid on the external nares and the rats were allowed to aspirate it.

Drug Administration. Each drug was given by intraperitoneal injection once a day for 4 or 8 days, starting 1 day before the first intranasal instillation of ETN (Fig 1). Indomethacin (2 or 4 mg/kg body weight per day) and DEX (4 or 8 mg/kg body weight per day) were injected for 4 days (3 days of ETN instillations), and EM (100 mg/kg body weight per

From the Department of Otorhinolaryngology, Mie University School of Medicine, Tsu, Mie, Japan.

CORRESPONDENCE — Takeshi Shimizu, MD, Dept of Otorhinolaryngology, Mie University School of Medicine, 2-174 Edobashi, Tsu, Mie 514, Japan.

Experiment A (3 days of endotoxin instillations)



Experiment B (7 days of endotoxin instillations)

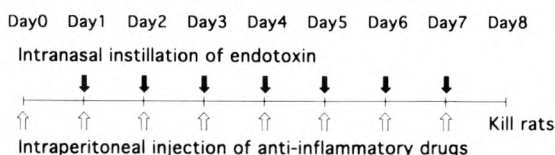


Fig 1. Experimental designs of 3 and 7 days of endotoxin instillations and drug administration.

day), ABPC (200 mg/kg body weight per day), and CET (200 mg/kg body weight per day) were given for 4 or 8 days (3 or 7 days of ETN instillations). For the injection, IND (Sigma Chemical Co) was dissolved in 1% sodium bicarbonate. Dexamethasone and each antibiotic drug were given in the form prescribable for injection: DEX sodium phosphate formulated as Decadron (Banyu Pharmaceutical Co, Tokyo, Japan), EMLactobionate as Erythrocin (Dainippon Pharmaceutical Co, Osaka, Japan), ABPC sodium as Viccillin (Meiji Pharmaceutical Co, Tokyo), and CET sodium as Keflin (Shionogi Pharmaceutical Co, Osaka).

Tissue Preparations. At 24 hours after the last instillation, five rats in each group were painlessly sacrificed with an intraperitoneal overdose of sodium pentobarbital. The head of each rat was removed and fixed in 10% neutral-buffered formalin containing 0.5% cetylpyridinium chloride for 24 hours, then decalcified in 5% trichloroacetic acid for 5 days. The nasal cavity was transversely sectioned at the level of the incisive papilla of the hard palate, and the tissue block was embedded in paraffin. Five-micron-thick paraffin sections were stained with Alcian blue (pH 2.6)-periodic acid-Schiff and hematoxylin (AB-PAS-H).

Morphometry. The percent area of AB-PAS-stained mucosubstance in the surface epithelium was determined with the image analyzer (SP 500, Olympus, Tokyo). The area of nasal epithelium was outlined, and the image analyzer determined the area of AB-PAS-stained mucosubstance within this reference area. The percent area of mucosubstance per epithelial area was calculated over 2 mm (1 mm of each side of nasal septum $\times$ 2) of the basal lamina at the center of the septal cartilage. Since the measured area of mucosubstance changes in the oblique section, the percent area of mucosubstance was used as a parameter of intraepithelial mucus production.

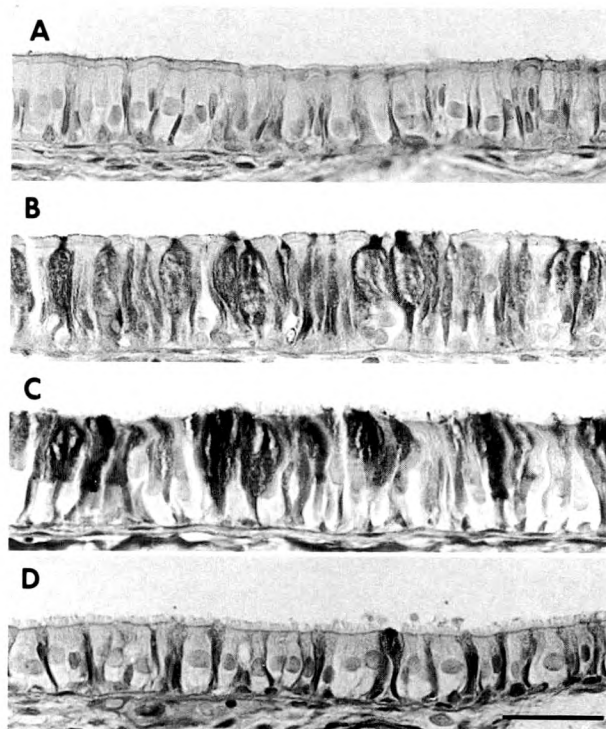


Fig 2. Effects of 0.1 mg of endotoxin instillation on nasal septal epithelium. A) Untreated control. B,C) Hypertrophic and metaplastic changes of goblet cells after B) 3 days and C) 7 days of endotoxin instillations. D) Saline-treated control (7 days of instillations). Bar — 30 μ m.

Statistics. All data are expressed as mean $\pm$ SD. Comparisons were performed with a Wilcoxon test. Probability values of $p < .05$ were considered significant.

RESULTS

Figure 2 illustrates the effects of 0.1-mg ETN instillation on nasal septal epithelium. Only a few goblet cells were observed in untreated controls or saline-instilled controls. The number of hypertrophic goblet cells and the amount of intraepithelial mucus increased after ETN instillations. Taller epithelium, hypertrophy of the cytoplasmic component, and submucosal inflammatory cell infiltration were also observed in ETN-instilled animals; however, we did not observe actual nasal discharge in these animals. Quantitative changes in the percent area of AB-PAS-stained mucosubstance are illustrated in Fig 3. The percent area of mucosubstance increased after ETN instillation and peaked after 3 days of instillations. No significant difference was observed between 3 and 7 days of instillations.

Figure 4A illustrates the effects of intraperitoneal injections of IND and DEX after 3 days of ETN instillations. Indomethacin and DEX inhibited ETN-induced hypertrophic changes of goblet cells, and quantitative study of the percent area of mucosub-

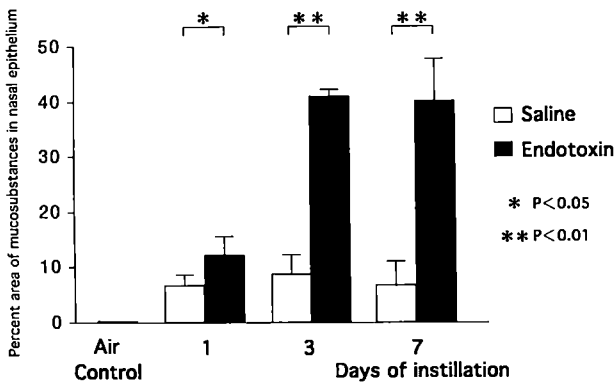


Fig 3. Percent area of mucosubstance in nasal epithelium after endotoxin instillations ($n = 5$). Endotoxin induces significant increase of epithelial mucosubstance, compared with saline exposure. "Air control" represents untreated animals.

stance shows the significant inhibition of intraepithelial mucus production in IND- or DEX-treated rats.

Figure 4B illustrates the effects of intraperitoneal injections of antibiotics — EM, ABPC, and CET — after 3 days of ETN instillations. The quantitative study of the percent area of mucosubstance shows the significant inhibition of intraepithelial mucus production in EM-treated rats; however, no significant inhibition was observed in ABPC- or CET-treated rats.

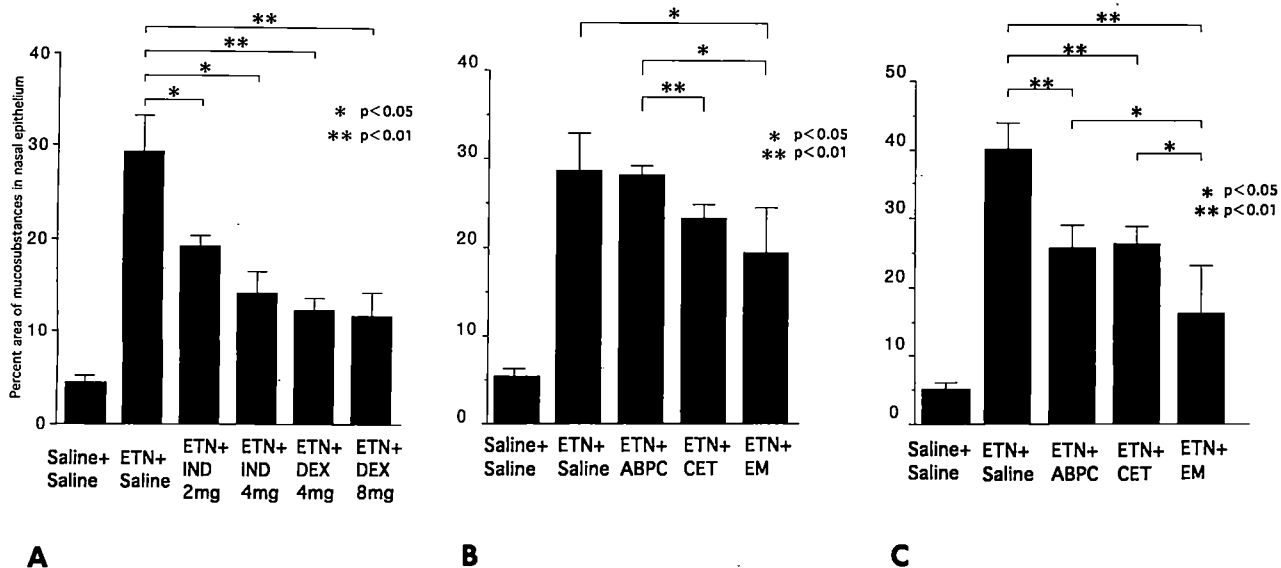
Since long-term EM treatment has been reported to be effective for patients with chronic airway inflammation, the effects of these antibiotics after 7 days of ETN instillations were also examined (Fig

4C). All these antibiotics significantly inhibited ETN-induced hypertrophic changes of goblet cells and intraepithelial mucus production, and when compared with ABPC and CET, EM had greater inhibitory effects on intraepithelial mucus production.

DISCUSSION

We produced hypertrophic and metaplastic changes of goblet cells in rat nasal respiratory epithelium by the intranasal instillation of ETN.⁵ A significant increase in the number of hypertrophic goblet cells has been observed in the nasal septal epithelium transversely sectioned at the level of the incisive papilla at 24 hours after 3 or 7 days of 0.1-mg ETN instillations. This is a good animal model for studying goblet cell hyperplasia/metaplasia and intraepithelial mucus production. This specific model was used to study the effects of IND, DEX, and EM on ETN-induced intraepithelial mucus production.

The mechanism by which ETN induced hypertrophic and metaplastic changes of goblet cells is unclear. It may have a direct effect on the epithelial cells, and it may also affect them indirectly through the activation of inflammation. The molecular mechanisms of ETN-induced inflammation have become apparent little by little. It has been reported that ETN stimulated the secretion of interleukin (IL)-1 and tumor necrosis factor from macrophage,⁸ and it induced the production of IL-6, IL-8, and granulocyte/macrophage colony stimulating factor from nasal fibroblasts.⁹ These inflammatory cytokines activate



A

B

C

Fig 4. Effects of intraperitoneal injections. A) Effects of indomethacin (IND; 2 to 4 mg/kg body weight) and dexamethasone (DEX; 4 to 8 mg/kg body weight) after 3 days of endotoxin (ETN) instillations ($n = 5$). Indomethacin and DEX significantly inhibited ETN-induced intraepithelial mucus production. B) Effects of erythromycin (EM), aminobenzylpenicillin (ABPC), and cephalothin (CET) after 3 days of ETN instillations ($n = 5$). Significant inhibition of intraepithelial mucus production was observed in EM-treated rats. C) Effects of EM, ABPC, and CET after 7 days of ETN instillations ($n = 5$). All these antibiotics significantly inhibited intraepithelial mucus production, and when compared with ABPC or CET, EM had greater inhibitory effect.

inflammation and induce the production of inflammatory mediators, which in turn may stimulate intraepithelial mucus production.

In the present study, intraperitoneal injection of anti-inflammatory drugs — IND or DEX — significantly inhibited ETN-induced intraepithelial mucus production. Indomethacin, an inhibitor of cyclooxygenase products of arachidonic acid, may act by inhibiting prostaglandin formation. Dexamethasone, a glucocorticoid, has several anti-inflammatory actions. Lundgren et al¹⁰ reported that a portion of the inhibitory effect of DEX on airway mucus secretion was related to the induction of the synthesis of the phospholipase inhibitor lipocortin, which inhibited arachidonic acid release and eicosanoid formation. These results raise the possibility that the inhibitory effects of IND and DEX on ETN-induced intraepithelial mucus production are partly attributable to the inhibition of arachidonic acid products.

Other inflammatory mediators and inflammatory cells are also responsible for these pathologic changes. Neutrophil depletion significantly inhibits ETN-induced mucus production,⁵ and neutrophil elastase induces mucus production and secretory cell metaplasia in tracheobronchial epithelium of the hamster^{3,11} and the rat.⁴ These results suggest that neutrophils and neutrophil-derived mediators play important roles in mucus production, and the anti-inflammatory effects of DEX included inhibitory effects on infiltrating neutrophils. Another possible action of DEX may be direct inhibitory effects on epithelial secretory cells. Shimura et al¹² reported that DEX inhibited the mucus production from isolated feline tracheal submucosal glands.

The clinical effectiveness of EM treatment has been reported for patients with chronic airway disease; however, the mechanism by which EM controls chronic inflammation is still unclear. The efficacy of this treatment may be attributed to the anti-inflam-

matory effects of EM rather than antibacterial ones, because 1) "low-dose" treatment, only half of the usual dose, resulted in good effects, 2) "long-term" treatment was required, and 3) this treatment was also effective for patients with pathogens insensitive to EM. Many investigators have reported anti-inflammatory actions of EM, which included 1) inhibitory effects on neutrophils,¹³ 2) modulation of the function of lymphocytes and macrophages,^{14,15} 3) inhibition of cytokine production,¹⁶ and 4) inhibition of elastase production from *Pseudomonas aeruginosa*.¹⁷

Mucus hypersecretion associated with hypertrophy, hyperplasia, and metaplasia of epithelial secretory cells is a major characteristic of chronic airway disease, and the clinical effectiveness of EM treatment is represented by a significant reduction of the amount of secreted mucus, sputum, and rhinorrhea. Goswami et al¹⁸ reported that EM had a direct inhibitory effect on mucus secretion from cultured epithelial cells in vitro. In the present study, the in vivo effects of EM on intraepithelial mucus production were examined, and intraperitoneal injection of EM significantly inhibited ETN-induced mucus production in rat nasal epithelium. Since other antibiotics, ABPC and CET, also had inhibitory effects on mucus production, a part of its inhibitory effects may be related to antibacterial action by preventing secondary bacterial infection. However, EM had greater inhibitory effects on mucus hypersecretion, compared with ABPC and CET. These additional inhibitory effects of EM may be caused by direct effects on the epithelial secretory cells, and the indirect effect through modulating the inflammation.

All these results support the effectiveness of clinical use of IND and DEX in the treatment of patients with chronic airway disease who are suffering from mucus hypersecretion. Antibiotics such as EM, ABPC, and CET will be also effective, probably by preventing secondary bacterial infection, and EM has additional inhibitory effects on mucus hypersecretion.

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NEONATAL HEARING SCREENING

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ORIGIN AND DISTRIBUTION OF NADPH DIAPHORASE-POSITIVE NERVES IN RAT NASAL MUCOSA

SEAYUONG JEON, MD

JINPYEONG KIM, MD

EUIGEE HWANG, MD

CHINJU, KOREA

The aim of this study was to localize the distribution of (reduced) nicotinamide-adenine dinucleotide phosphate (NADPH) diaphorase-positive nerves in the rat nasal mucosa by NADPH diaphorase histochemistry, and to determine its origin by utilizing retrograde tracing with Fluoro-Gold (FG). Fine varicosities of NADPH diaphorase-positive nerve fibers were distributed around blood vessels (arterioles in particular), submucosal glands, and the subepithelial layer of the nasal mucosa. Most of the ganglion cells and nerve fibers in the sphenopalatine ganglion, and a few ganglion cells in the trigeminal ganglion, were stained by NADPH diaphorase, but no NADPH diaphorase-positive ganglion cells were found in the superior cervical ganglion. Retrograde tracing with FG and co-localization of NADPH diaphorase demonstrated that the FG-labeled ganglion cells in the sphenopalatine ganglion were NADPH diaphorase-positive, but the FG-labeled ganglion cells in both the trigeminal and the superior cervical ganglia were NADPH diaphorase-negative. In conclusion, NADPH diaphorase-positive nerves distribute around blood vessels, around submucosal glands, and in the subepithelial layer of the rat nasal mucosa, and their origin is the sphenopalatine ganglion. These findings imply that nitric oxide may be co-localized to the cholinergic innervation and be involved in vasomotor and secretomotor control of the nasal mucosa.

KEY WORDS — Fluoro-Gold, NADPH diaphorase histochemistry, nasal mucosa, nitric oxide, retrograde tracing, sphenopalatine ganglion.

INTRODUCTION

Nitric oxide (NO), first identified as the chemical responsible for endothelium-derived relaxing factor activity, now appears to be a neurotransmitter-like neuronal messenger in the brain and peripheral nervous system.^{1,2} Nitric oxide relaxes the smooth muscle of blood vessels, intestines, and the genitourinary tract.³ In addition to classic cholinergic and adrenergic innervation of the airway, nonadrenergic noncholinergic (NANC) innervations contribute to both bronchodilation and bronchoconstriction, vasodilation and vasoconstriction, and glandular secretion. Inhibitory NANC (i-NANC) nerves relax airway smooth muscle, dilate vessels, and stimulate glandular secretion.⁴ Current evidence suggests that vasoactive intestinal peptide (VIP) may mediate i-NANC effects in some species, but that NO is also involved.^{5,6} In the human airway, the predominant i-NANC transmitter appears to be NO.⁷ Thus, given the possible importance of NO to airway function, it is necessary to characterize the distribution and origin of the innervation in which NO is formed and acts as a neuronal messenger in the airway.

Attempts at complete description of the location and amount of NO acting as a neurotransmitter have been hampered by its short half-life and gaseous nature. Nitric oxide-mediated neurons can be local-

ized indirectly by immunohistochemical staining for the enzyme responsible for NO synthesis, NO synthase (NOS),⁸ or by histochemical staining for the enzyme (reduced) nicotinamide-adenine dinucleotide phosphate (NADPH) diaphorase, which is a co-enzyme in NO synthesis.^{9,10} NADPH diaphorase staining occurs as a blue precipitate with nitroblue tetrazolium (NBT).

The aim of this study was to localize the distribution of NADPH diaphorase-positive nerves in rat nasal mucosa by NADPH diaphorase histochemistry, and to determine the origin of the innervation by utilizing retrograde tracing with Fluoro-Gold (FG).

MATERIALS AND METHODS

NADPH Diaphorase Histochemistry. Three white rats weighing 250 to 300 g were used. The animals were anesthetized with intraperitoneally administered ketamine hydrochloride (75 mg/kg) and xylazine hydrochloride (10 mg/kg) and then intravascularly perfused with 2% paraformaldehyde in 0.1 mol/L phosphate buffer (PB; pH 7.2). The nasal septum was removed, cut into small blocks, and kept in the same fixative at 4°C for 4 hours. Specimens were washed in PB, saturated overnight at 4°C with 25% sucrose in PB, frozen in liquid nitrogen, and cut into sections of 8- μ m thickness on a cryostat. The cryostat sections

From the Department of Otolaryngology, GyeongSang National University Hospital, Chinju, Korea.

CORRESPONDENCE — SeaYuong Jeon, MD, Dept of Otolaryngology, GyeongSang National University Hospital, Chilamdong 92, Chinju, 660-702, Korea.

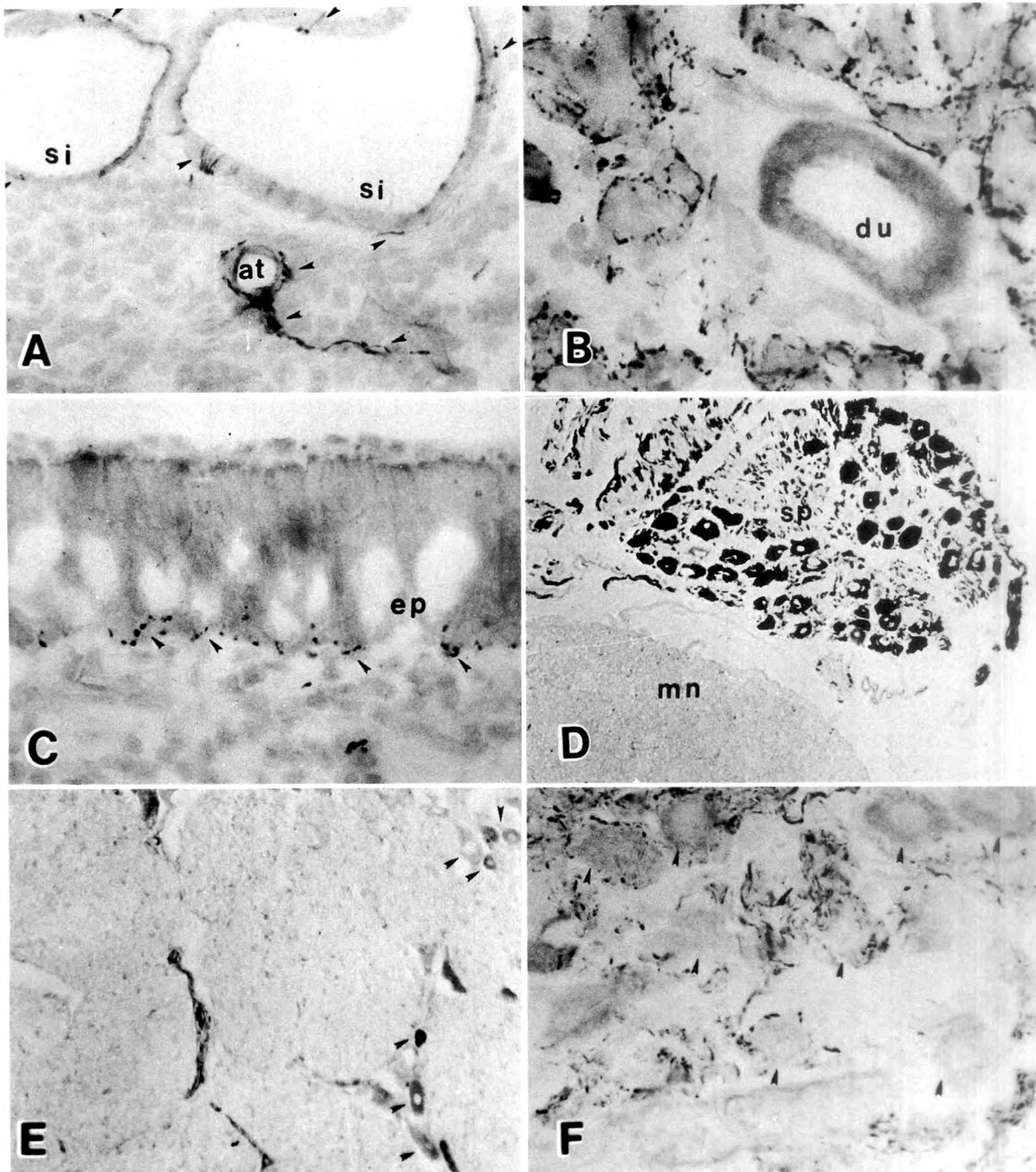


Fig 1. A-C) Distribution of NADPH diaphorase-positive nerves in rat nasal mucosa (original $\times 400$). A) NADPH diaphorase-positive reaction is localized in nerve fibers (arrowheads) distributed around small arteriole (at) and along wall of sinusoids (si). B) Numerous fine varicosities of NADPH diaphorase-positive nerve fibers are distributed around submucosal glands, but there is no positive innervation around duct (du). C) Fine varicosities of NADPH diaphorase-positive nerve fibers (arrowheads) are distributed beneath lining epithelium (ep). D-F) Distribution of NADPH diaphorase-positive neurons in various ganglia innervating nasal mucosa (original $\times 100$). D) Most of ganglion cells and nerve fibers are stained intensely to NADPH diaphorase in sphenopalatine ganglion (sp). Maxillary nerve (mn) adjacent to sphenopalatine ganglion has no positive reaction. E) Some NADPH diaphorase-positive ganglion cells (arrowheads) and nerve fibers are localized in trigeminal ganglion. F) None of ganglion cells (arrowheads) are stained to NADPH diaphorase, but fine varicosities of NADPH diaphorase-positive nerve endings are distributed around ganglion cells in superior cervical ganglion.

were dried and hydrated in PB. After washing with PB, the sections were incubated in a mixture of NADPH (1 mg/mL), NBT (0.1 mg/mL), and 0.2% Triton X-100 in PB for 30 minutes at 37°C. Then,

sections were washed with PB, counterstained with methyl green, and mounted with buffered glycerol.

As a tissue control, specimens from the cerebellum

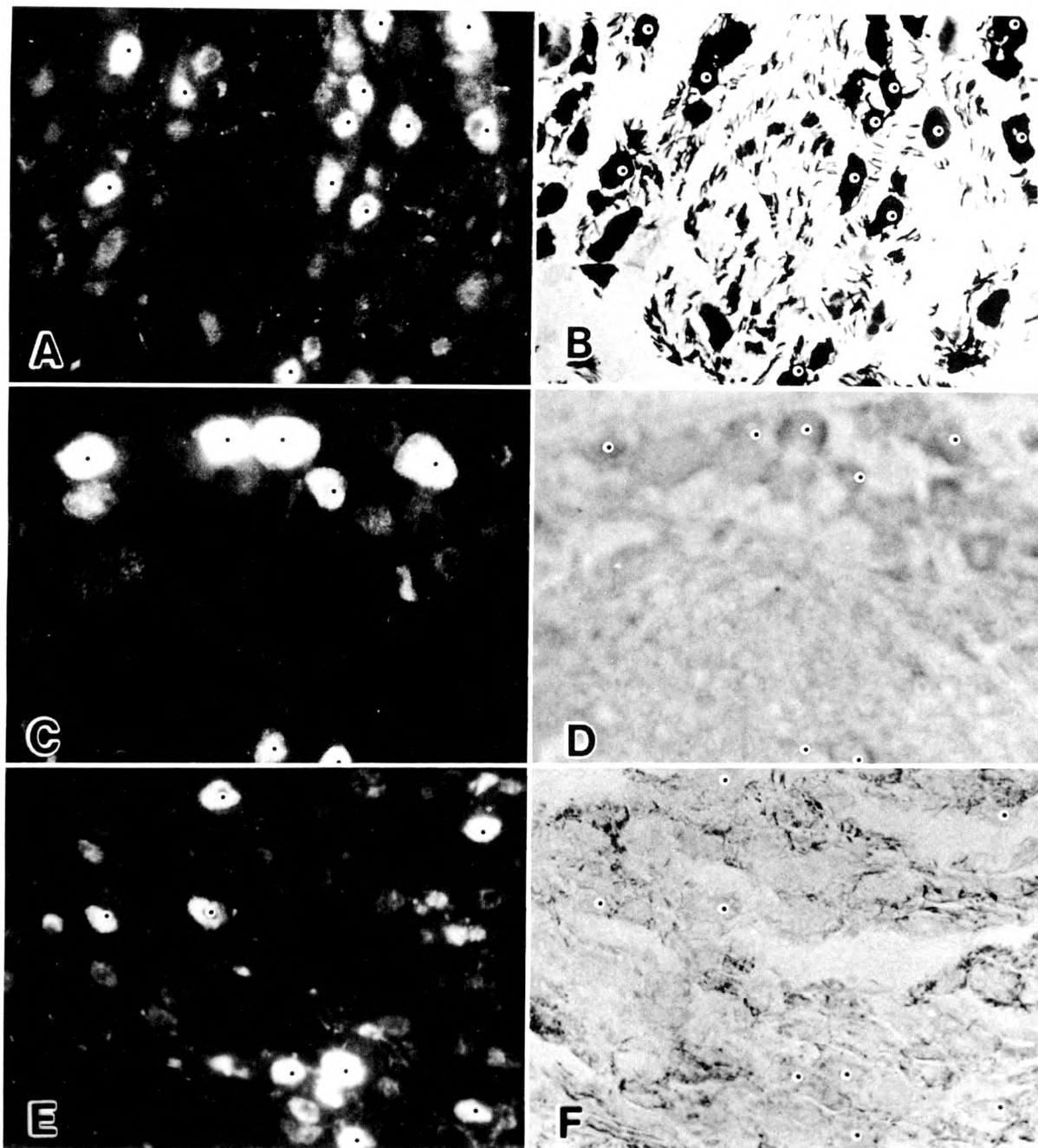


Fig 2. NADPH diaphorase and Fluoro-Gold (FG) co-localization (original $\times 100$). A,B) FG-labeled ganglion cells in sphenopalatine ganglion are NADPH diaphorase-positive. Dots indicate some of double-labeled ganglion cells. C-F) FG-labeled ganglion cells in trigeminal (C,D) and superior cervical (E,F) ganglia have no evidence of NADPH diaphorase reaction. Dots indicate same ganglion cells in each set of photomicrographs.

were processed, and as a negative control for the NADPH diaphorase reaction, additional cryostat sections were processed in media from which the substrate had been omitted.

Retrograde Tracing With FG and Co-localization of NADPH Diaphorase. Another three rats were anesthetized, and a fresh wound was made in the nasal mucosa by scratching with a cotton swab. Ten microliters of 5% FG was instilled into the nasal

cavity. Seven days later, each rat was reanesthetized and intravascularly perfused with fixative. The trigeminal ganglion, sphenopalatine ganglion, and superior cervical ganglion were removed and then processed for cryostat sectioning as previously described. Cryostat sections were dried, washed with PB, and coverslipped with glycerin-phosphate-buffered saline as a mounting medium. Each section was examined for tracer fluorescence with a wide-band ultraviolet excitation filter (excitation, 323 nm; emission,

408 nm) and photographed.

The coverslips were removed and the tissue sections were then processed for NADPH diaphorase histochemistry as previously described. Tissue sections were examined for the presence of NADPH diaphorase-positive ganglion cells and photographed. Each set of photomicrographs was then analyzed for the double-labeled ganglion cells that were both NADPH diaphorase-positive and labeled with FG.

RESULTS

NADPH Diaphorase Histochemistry. Fine varicosities of NADPH diaphorase-positive nerve fibers were distributed around blood vessels (arterioles in particular), around submucosal glands, and in the subepithelial layer of the nasal mucosa (Fig 1A-C). Most of the ganglion cells and nerve fibers in the sphenopalatine ganglion were stained by NADPH diaphorase, and a few ganglion cells in the trigeminal ganglion were stained by NADPH diaphorase. No NADPH diaphorase-positive ganglion cells were found in the superior cervical ganglion, but some NADPH diaphorase-positive nerve endings were found around the ganglion cells (Fig 1D-F).

In the tissue control of cerebellar cortex, molecular and granular cells were stained by NADPH diaphorase. In the negative control sections, NADPH diaphorase activity was not observed.

Retrograde Tracing With FG and Co-localization of NADPH Diaphorase. Intensely FG-labeled ganglion cells were found in all the ganglia. The FG-labeled ganglion cells in the sphenopalatine ganglion were found to be NADPH diaphorase-positive, but the FG-labeled ganglion cells in both the trigeminal and the superior cervical ganglia were found to be NADPH diaphorase-negative (Fig 2). Thus, topographically distinct ganglion cells double-labeled by FG and NADPH diaphorase were localized to the sphenopalatine ganglion.

DISCUSSION

This study demonstrates that NADPH diaphorase-positive nerves are distributed around blood vessels, around submucosal glands, and in the subepithelial layer of the rat nasal mucosa, and that their origin is the sphenopalatine ganglion. Because NO has only recently emerged as the major NANC neurotransmitter in the peripheral nervous system, the study of NO in the nasal airway is very new. Hanazawa et al¹¹ reported the distribution of NADPH diaphorase-positive nerve fibers in the rat nasal mucosa in 1993, and our findings agree with their description. It has been reported that NOS in blood vessels is restricted

to the endothelial layer, but in the cerebral arteries⁸ and arteries in the penis,¹² NOS is localized to the nerve plexus in the outer, adventitial layer. In the rat nasal mucosa, NADPH diaphorase-positive nerve fibers surround the arterioles and run along the wall of sinusoids as in the penis. However, histochemical staining for NADPH diaphorase is not specific for NOS. The localization of NOS by immunohistochemical staining should be used to confirm our findings.

The origin of NADPH diaphorase-positive nerves in the nasal mucosa has not previously been reported. This study determined the origin of NADPH diaphorase-positive nerves in the rat nasal mucosa by utilizing a combination of retrograde tracing with FG and NADPH diaphorase histochemistry. Hanazawa et al¹¹ suggested that most of the labeled fibers originated from the pterygopalatine ganglion and that the rest of them originated from the trigeminal ganglion, for many neurons in the pterygopalatine ganglion were labeled, and a few neurons were stained in the trigeminal ganglion. Their suggestion was not proven by retrograde tracing or denervation experiments. We observed that a few ganglion cells in the trigeminal ganglion were positive for NADPH diaphorase, but none of them were FG-labeled. We examined the maxillary nerve, and could not find any NADPH diaphorase-positive nerve fibers. We suggest that a population of NADPH-diaphorase-positive ganglion cells in the rat trigeminal ganglion might be localized to the motor root of the ganglion by which the mandibular division is joined.

It has been demonstrated that NOS is localized to the cell bodies and fibers of the myenteric plexus, the parasympathetic innervation of the intestine.⁸ The origin of the NADPH diaphorase-positive neurons innervating the urinary bladder has been determined to be the major pelvic ganglia, the parasympathetic innervation of the bladder.¹³ It has been suggested that VIP and NO may be co-localized and co-released from cholinergic nerves and act as functional antagonists of cholinergic constriction of the airway smooth muscle.⁴ The origin and distribution of NADPH diaphorase-positive nerves in the rat nasal mucosa appears to coincide with the cholinergic innervation, which originates from the sphenopalatine ganglion and is distributed around blood vessels, around submucosal glands, and beneath the surface epithelium. Of course, this suggestion should be confirmed by double labeling with NADPH diaphorase histochemistry and choline acetyltransferase immunohistochemistry. Unfortunately, the physiological role of NO in the nasal mucosa is still unknown. However, the origin and distribution of NADPH diaphorase nerves in the nasal mucosa suggest that NO may serve a role

in vasomotor control and glandular secretion.

In conclusion, NADPH diaphorase-positive nerves are distributed around blood vessels, around submucosal glands, and in the subepithelial layer of the rat

nasal mucosa, and their origin is the sphenopalatine ganglion. These findings imply that NO may be colocalized to the cholinergic innervation and may be involved in vasomotor and secretomotor control of the nasal mucosa.

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IMPACT OF A LARYNGECTOMY ON QUALITY OF LIFE: PERSPECTIVE OF THE PATIENT VERSUS THAT OF THE HEALTH CARE PROVIDER

RANDAL A. OTTO, MD
VALERIE LAWRENCE, MD

ROBERT A. DOBIE, MD
CONNIE SAKAI, MSPA

SAN ANTONIO, TEXAS

This study retrospectively assesses the impact of laryngectomy on the quality of life of 46 patients as compared to the perception of the impact of laryngectomy of 13 health care providers (HCPs). Employing the "time trade-off" methodology, we assessed patient and HCP preferences and calculated estimated utilities. We found that 20% of patients would be willing to compromise anticipated life expectancy to preserve voice or preoperative quality of life. By comparison, 46% of the HCPs perceived that their patients would be willing to accept a reduced life span in order to preserve their larynx and quality of life. In conclusion, the percentage of HCPs who believed their patients would compromise survival was substantially higher than the percentage of actual patients who expressed this preference. This perception may influence physicians' attitudes toward recommending laryngeal preservation therapy for their patients. For most laryngectomy patients, treatments attempting laryngeal preservation, particularly if associated with compromised survival, may not be warranted.

KEY WORDS — laryngectomy, quality of life, time trade-off.

INTRODUCTION

Traditionally, the success of cancer treatment has been assessed by objective measures such as disease-free intervals, cure rates, and complication rates. Recently, however, there has been an increased awareness of the need to evaluate treatment effects on the individual's quality of life. Methodologies such as the quality-adjusted life year (QALY) and utility (a measurement of preferences or value) have been introduced as tools designed to take into account the quality, duration, and relative desirability of life associated with health care procedures or services.^{1,2} Unfortunately, relatively few studies involving specific treatment alternatives and their impact on the quality of life are available. Nonetheless, more costly treatment options are sometimes offered, not because of demonstrated survival advantages, but because of our perception that the standard treatment is more disabling or is associated with increased morbidity. This, indeed, may be the case for treatment of the patient with advanced laryngeal cancer where a laryngectomy, possibly combined with radiotherapy, is considered the "gold standard." However, because of the perception held by many laypersons and professionals that a laryngectomy is severely disabling, a great deal of interest exists in alternative treatments that will preserve the larynx.³⁻⁵ Notable among these has been the report by Wolf et al⁵ that induction chemotherapy followed by radiotherapy for patients

with advanced laryngeal cancers, while offering no survival advantage, resulted in laryngeal preservation in 64% of the surviving patients. However, for those patients whose larynges are preserved, several quality of life and cost issues emerge. These include the morbidity and cost of the chemotherapy, as well as the need for multiple endoscopies under general anesthesia in all patients to confirm a complete response. Patients with persistent neck disease will still require neck dissections, and those who fail at the primary site will require surgical salvage procedures with the associated costs and morbidities. Additionally, it has been our experience that some patients will become quite depressed with the prospect of recurrence and the concern that since the tumor was never actually removed, their cancer may still exist. Therefore, it is important that the underlying premise (that surgical removal of the larynx substantially compromises the quality of life of the laryngectomy patient) be evaluated for its validity. Additionally, if this premise is not universally true, then the factors influencing the laryngectomee's perception of quality of life must be identified.

This study attempts to address these concerns by assessing the impact of a laryngectomy in 46 post-laryngectomy patients and comparing it to the perception of 13 health care providers (HCPs) routinely treating laryngectomy patients by employing the "time trade-off" methodology to assess preferences

From the Departments of Otolaryngology-Head and Neck Surgery (Otto, Dobie, Sakai) and Medicine (Lawrence), The University of Texas Health Science Center at San Antonio, San Antonio, Texas.

CORRESPONDENCE — Randal A. Otto, MD, Dept of Otolaryngology-Head and Neck Surgery, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr, San Antonio, TX 78284-7777.

TABLE 1. CLINICAL, SOCIAL, AND EDUCATIONAL VARIABLES OF 46 LARYNGECTOMY PATIENTS

<i>Clinical Variables</i>	<i>Social Variables</i>	<i>Educational Variables</i>
Tumor stage (n = 28)*	Marital status	Education
I 2 (7%)	Preoperative	Level of education (n = 44)
II 4 (14%)	Married 33 (72%)	None 1 (2%)
III 10 (36%)	Single 3 (7%)	Elementary school 7 (16%)
IV 12 (43%)	Divorced 7 (15%)	High school 16 (36%)
Preoperative counseling	Separated 1 (2%)	Technical school 5 (11%)
Doctor 39 (85%)	Widowed 2 (4%)	College 8 (18%)
Speech pathologist 26 (57%)	Change	Graduate school 7 (16%)
Other patients 14 (30%)	Married 0 (0%)	Ability to read (n = 45) 44 (98%)
Adjuvant treatment 36 (78%)	Single 0 (0%)	Ability to write (n = 45) 43 (96%)
Surgical 19 (41%)	Divorced 2 (4%)	
Chemotherapy 3 (7%)	Separated 0 (0%)	
Radiotherapy 27 (59%)	Widowed 0 (0%)	
Postlaryngectomy complications 18 (39%)	Living arrangement	
Stenosis (stomal and/or esophageal) 8 (17%)	With spouse 28 (61%)	
Wound infection or fistula 4 (9%)	With family 7 (15%)	
Other 6 (13%)	With family and spouse 3 (7%)	
Interval since surgery (n = 44)	Alone 6 (13%)	
Range 1 to 216 mo	Other 2 (4%)	
Mean 40.2 mo	Change in living arrangement	
Oral communication 44 (96%)	(n = 45) 10 (22%)	
Electrolarynx 32 (70%)	Income decrease 13 (28%)	
Esophageal 2 (4%)		
Tracheal esophageal prosthesis 13 (28%)		
None 2 (4%)		
Postoperative verbal communication		
by any means 45 (98%)		

*Staging per Beahrs OH, Henson DE, Hutter RVP, Myers MH, eds. Manual for staging of cancer. American Joint Committee on Cancer. 3rd ed. Philadelphia, Pa: JB Lippincott, 1988:39-44.

and calculate utility and QALY values.^{1,6-8} An analysis of factors possibly contributing to altered utility and quality of life is also presented.

METHODS

PATIENT STUDY

Forty-six postlaryngectomy patients were personally interviewed by a neutral observer, and when possible, their medical records were reviewed. Thirty-one were patients treated and/or followed by the Department of Otolaryngology-Head and Neck Surgery at The University of Texas Health Science Center at San Antonio. Fifteen of the subjects were members of the local American Cancer Society. Patient ages ranged from 49 to 80 years old, with a mean age of 66.2. Forty (87%) patients were male; 6 (13%) were female. Thirty-seven (80%) patients were white, 7 (15%) were Hispanic, 1 was African-American, and 1 was Asian-American. Additional clinical, educational, and social variables potentially related to patient perceptions of health and quality of life or willingness to trade survival for quality of life are shown in Table 1.

Patient perceptions regarding current and preoperative quality of life and health were obtained with a modification of the linear analog rating scale.⁹ Each patient was asked to rate his or her health and quality of life preoperatively and at the time of the interview by using a 1 to 10 scale, with "10" representing the best and "1" representing the poorest end of the spectrum. Patient preferences were evaluated by means of the "time trade-off" methodology.⁶⁻⁸ Patients were asked, "Assume that surgery (laryngectomy) was known to provide 10 years of survival or life expectancy from the date of surgery. Would you have been willing to accept a somewhat shorter survival and undergone other surgical or nonsurgical treatment(s) that would have preserved your voice box? Yes/No." This was followed by the question "If yes, how many years out of the 10 years would you be willing to trade to maintain the quality of life you had prior to surgery? (Circle a number 1 through 10)." This was cross-checked with the following fill-in-the-blank question: "In other words, you believe ___ years of life with a voice box is equivalent to 10 years without." Assuming a 10-year life expectancy, the utility and QALY values were then calculated for

TABLE 2. HEALTH CARE PROVIDER RESPONSES

Age	Years in Practice	Subspecialty	Would Patient Accept Shorter Life Span?	
			Yes	Years Traded
32	1	ENT	X	4
33	2	ENT		
33	2	ENT	X	5
37	5	ENT		
38	1	ENT	X	2
47	16	ENT		
48	16	ENT		
56	22	ENT		
58	27	ENT	X	2
69	42	ENT	X	5
28	4	SP		
42	15	Rad-Onc		
46	17	Rad	X	2

ENT — otolaryngology—head and neck surgery, SP — speech pathology, Rad-Onc — radiation oncology, Rad — radiology.

each patient as follows.

$$\text{Utility} = 1 - \frac{(\text{Number of years traded})}{10}$$

$$\text{QALY} = \text{Utility} \times 10$$

Thus, a patient who indicated a willingness to trade 7 out of 10 years of life expectancy (estimated utility = 0.3) would have an estimated QALY = 0.3×10 or 3. In other words, 3 years with a larynx is estimated, given the above life expectancy assumption, to be equivalent to 10 years without a larynx for this patient.

HEALTH CARE PROVIDER STUDY

Twelve physicians and one speech pathologist routinely involved in the treatment of laryngeal cancer patients also completed a questionnaire attempting to assess their perception of the impact of a laryngectomy on their patients. Ages of the HCPs ranged from 28 to 69 years, with a mean age of 43.6 years. The number of years these individuals had practiced in their area of care ranged from 1 to 42 years, with a mean of 13 years (Table 2).

Questions similar to those posed to the patients were asked: "Assume that a laryngectomy was known to provide 10 years of survival from the date of surgery. From your perception as a health care provider, do you believe that your patient (average age 55 to 65) would have been willing to accept a somewhat reduced average life expectancy and undergo an alternative, nonsurgical treatment, ie, radiotherapy or chemotherapy, to preserve their larynx and allow

TABLE 3. LARYNGECTOMY PATIENTS' PERCEPTION OF HEALTH

	Those Willing to Trade (n = 9)	Those Not Willing to Trade (n = 37)	Difference
Preop	5.72	7.93	2.21 (p = .02)
Current	6.89	8.26	1.37 (p = .12)
Change	1.17 (p = .2510)	0.33 (p = .4466)	-0.84 (p = .40)

Data are mean scores on 1 to 10 scale (10 = best) assessing health.

them to maintain their quality of life? Yes/No." This was then followed by the question "If yes, how many years out of the 10 years do you think your patient would be willing to trade to maintain their quality of life and preserve their larynx? (Circle a number 1 through 10)." As with the patients, the following cross-check question was then presented: "In other words, you believe ___ years of life with a voice box is equivalent to 10 years without." Utility and QALY data were then calculated as described above.

STATISTICS

The primary dependent variables in this study were utility values determined by both patients and HCPs and patients' self-assessment scores for preoperative and postoperative quality of life and health status. Since utility scales were bounded between 0 and 1, and quality of life scores were bounded between 1 and 10, none of these could be considered normally distributed, given expected ceiling effects. However, change (postoperative minus preoperative) scores for patient self-assessment were expected to be normally distributed. Paired *t*-tests were performed for change in self-assessment scores, both for patients who were willing, and for those who were unwilling, to trade life expectancy for laryngeal preservation. Differences between these two groups were tested for statistical significance with an unpaired *t*-test.

Forward stepwise logistic regression analysis was performed, with willingness to trade used as the nominal dependent variable. Due to the statistical constraints imposed by the number of patients interviewed, the variables selected for analysis were limited to those that the authors thought would most likely predict the patients' decision to trade (preoperative counseling, tumor stage, patient age, gender, race, marital status, living arrangements, perceived preoperative health, perceived preoperative quality of life, and education). Tumor stage was subsequently eliminated from analysis due to insufficient data.

TABLE 4. LARYNGECTOMY PATIENTS' PERCEPTION OF QUALITY OF LIFE

	Those Willing to Trade (n = 9)	Those Not Willing to Trade (n = 37)	Difference
Preop	8.67	8.45	0.22 (p = .75)
Current	6.78	7.72	0.94 (p = .30)
Change	-1.89 (p = .0934)	-0.73 (p = .0313)	1.16 (p = .16)

Data are mean scores on 1 to 10 scale (10 = best) assessing quality of life. Utility and quality-adjusted life years value calculations were based on estimated 10-year life expectancy.

RESULTS

PATIENT RESPONSES

Health. The patients' perceptions of health preoperatively and at the time of the interview ranged from 1 to 10, with mean scores of 7.49 and 7.99, respectively, representing a mean improvement of 0.49 (range -8 to 9). Patients who would have been willing to trade off life expectancy to preserve their larynx started at a lower perceived health status preoperatively (5.72 versus 7.93, $p = .02$) than patients unwilling to trade. Both groups of patients showed improvements in perceived health status posttreatment (mean change of 1.17 for traders, 0.33 for nontraders), but these changes were not statistically significant (Table 3). An unpaired t -test showed that the change scores did not differ between the two groups.

Quality of Life. The perception of quality of life preoperatively and at the time of the interview with the laryngectomy patients ranged from 1 to 10, with mean scores of 8.49 and 7.53, respectively. The range of changes occurring in the patients' quality of life after laryngectomy varied from -8 to 4, with a mean change for the worse of -0.96. Patients who would have been willing to trade off life expectancy to preserve their larynx had experienced larger decrements in their quality of life (-1.89 average) as compared to those patients unwilling to trade (-0.73 average; Table 4).

Paired t -test analysis of quality of life responses revealed that laryngectomy patients, both those who indicated a willingness to compromise survival and those who did not, experienced a significant reduction in perceived quality of life ($p = .0934$ and $.0313$, respectively). However, the reduction of quality of life experienced by the patients willing to trade was not statistically different from that of those unwilling to trade ($p = .16$; Table 4).

Time Trade-off Responses. Thirty-seven (80.4%) of the 46 patients interviewed would not be willing to

TABLE 5. "TIME TRADE-OFF" RESPONSES OF PATIENTS AND HEALTH CARE PROVIDERS

	Average No. of Years Traded	Average Utility	Average QALY
Patients			
All (n = 46)	1.1	0.89	8.9
Those willing to trade (9/46)	5.6	0.44	4.4
Health care providers			
All (n = 13)	1.54	0.85	8.5
Those willing to trade (6/13)	3.33	0.67	6.7

Utility and quality-adjusted life years (QALY) value calculations were based on estimated 10-year life expectancy.

trade off any life expectancy for quality of life or voice preservation. Nine patients (19.6%) indicated a willingness to trade 2 to 10 years of their anticipated life expectancy in order to maintain their laryngeal voice and preoperative quality of life. Acceptable time traded averaged 1.1 years for the entire laryngectomy cohort; however, this increased to an average of 5.6 years when limited to those patients who indicated they would have been willing to trade. Two patients initially indicated a willingness to trade; however, when subsequently asked how many years out of the anticipated 10 years of life expectancy they would trade, they decided not to trade any years (Table 5).

Logistic regression analysis, limited only to those factors that would be available preoperatively (preoperative counseling, patient age, gender, race, marital status, living arrangements, perceived preoperative health, perceived preoperative quality of life, and education) demonstrated that only a patient's perceived poor preoperative health was associated with an increased likelihood of the patient's being willing to trade ($p = .0121$).

HEALTH CARE PROVIDER RESPONSES

Time Trade-off Responses. Forty-six percent of the HCPs perceived that their patients would be willing to accept a reduced survival in order to preserve their larynx and quality of life. The average reduction in survival recorded from all HCPs was 1.54 years, although those who perceived that their patients would trade indicated that their patients would trade 2 to 5 years, with a mean of 3.33 years (Tables 2 and 5).

DISCUSSION

Relatively few studies address the consequences of laryngectomy on quality of life.¹⁰⁻¹⁴ Although it has been well established that loss of voice is disabling, quantitative assessment of the impact of laryngectomy as compared to HCPs' perceptions or that

of alternative treatments has received little attention. McNeil et al¹⁵ attempted to address this, in part, by applying the concept of utility to laryngeal cancer treatment. On the basis of volunteers' responses, McNeil et al calculated that 20% of the subjects would have chosen radiotherapy (with an assumed reduced survival) over surgery in an attempt to maintain their voices. Although these results appear similar to ours, we were unfortunately, unable to make any direct comparisons of the responses from our patients to those of McNeil et al, due to unavailability of their data (personal communication, B. J. McNeil, 1994). However, some important differences in the two study designs should be noted. First of all, the McNeil et al study involved primarily healthy middle and upper management executives who were not actually facing a laryngectomy, and indeed may not have ever known anyone who had undergone a laryngectomy. Thus, as noted by other authors, the imaginary health state posed to these subjects may have been evaluated as less desirable than if it had actually been experienced.^{16,17} The subjects in the McNeil et al study also differed in terms of socioeconomic status from the general population afflicted by laryngeal cancer.¹⁸⁻²⁰ Additionally, the average age of the volunteers in the McNeil et al study was 40 years, which is considerably younger than that of most laryngectomy patients, and they were given an expected survival of 25 years, which is considerably greater than one would give for an average laryngectomy patient.^{15,18,21} Given that the mean age of our patients was 66.2 years, and that the average life expectancy of a 65-year-old smoker is less than 10 years,²² this would further suggest that the volunteers in their study represent poor "proxy judges." Likewise, the HCP portion of our study suggests that even those with extensive knowledge of the treatment are poor "proxy judges," with 46% of HCPs (versus 20% of patients) indicating that they felt their patients would compromise survival to preserve voice and quality of life. This fact becomes increasingly important as the trend continues to recommend potentially more costly organ preservation therapy with no proven survival advantage.

The results of our study suggest that the perception of HCPs may diverge considerably from that of the patient. This is crucial, since we as HCPs often influence the patient's ultimate decision. Overall, the quality of life for most laryngectomy patients, although reduced, is not so severely affected that they would be willing to compromise survival to avoid a laryngectomy. This is reflected by the fact that 80% of patients interviewed were unwilling to compromise any life expectancy to avert the consequences of a laryngectomy on quality of life. However, for

certain individuals, a laryngectomy does severely compromise the patient's perception of quality of life. For these few patients, the utility of life following the laryngectomy decreased by over 50% (1.0 versus 0.44). Given a life expectancy of 10 years, these patients would have traded 56% of their life expectancy to have preserved their larynx and preoperative quality of life. It is these patients we must learn to identify preoperatively in order to provide optimal treatment recommendations.

Although, prior to this study, we felt we could predict those patients who would have been willing to trade, this has not been the case. Our analysis was only able to identify a patient's perceived poor preoperative health as a weak preoperative predictor of a willingness to trade. Factors influencing communication proved to have no predictive use. Indeed, one of our patients, who we all felt would trade because he could not read, write, speak English, or communicate orally, and came from a poor socioeconomic situation with little family support, was not willing to compromise survival. This further emphasizes that improved survival remains more important than laryngeal preservation, or any other factors we as HCPs may consider, and suggests that further studies are necessary to identify factors that influence a patient's quality of life to the extent that laryngeal preservation is warranted. Only with this information can cost-effective treatment plans be pursued that will provide the best care for the individual patient.

Several potential sources of bias may exist with this study. Probably most important is the fact that this was a retrospective study and that all our patients had undergone a laryngectomy and survived. This is important for a variety of reasons. Since all were survivors, they were never confronted with treatment failure. Therefore, for some it might have been easy to grieve over the loss of their larynx and theoretically consider accepting a shorter survival to preserve their larynx. Others, however, may have been subject to cognitive dissonance. To contemplate that they could have done something to save their voice may not have been easy for them to consider without also implying that they had made the wrong decision. Recall bias, in which patients rate a past event far more positively retrospectively than they do the same event prospectively, may have also biased our results. This may have been further exaggerated in those patients who had survived for many years (up to 18 years) without a larynx and thus had more time to acclimate to the loss. A better design would prospectively interview individuals who have selected laryngeal preservation as well as those selecting laryngectomy. This would allow further insight into the impact of the morbidity

of alternative treatment modalities (ie, chemotherapy, radical radiotherapy), the uncertainty of tumor removal, the necessity of repeated endoscopies, treatment failures, and ultimate surgical salvage for those patients who eventually face this option.

Another weakness of this study is that the questions posed to the HCPs probably should have included an inquiry as to the percentage of patients who they felt would have traded. This would allow for a more accurate comparison with the percentage of patients willing to trade. Likewise, questions to the patients regarding willingness to trade probably should incorporate increments of less than 1 year. This flaw was recognized when we encountered 2 patients who indicated a willingness to trade off anticipated life expectancy, but responded "none" when asked specifically how many years out of 10 years of anticipated life expectancy they would trade. When asked the cross-check question, "In other words, you believe ___ years of life with a voice box is equivalent to 10 years without," both responded with "10." This may reflect a change in attitude once faced with a specific trade-off or a willingness to trade less than 1 year.

One can also criticize the use of an assumed 10 years of life expectancy. Ideally, one should use actuarial tables matched to each patient. Unfortunately, we were unable to identify any such tables that take into consideration the fact that the individual already has been treated for laryngeal cancer and has a reasonable chance of developing related cancers. Thus, an assumed survival of 10 years was selected on the basis of an anticipated mean for age and sex of our patient population. If one considers that in most circumstances life expectancy was exaggerated, this

may have had an impact upon the patient responses. Since proximal years are considered by patients to be more valuable than distal years,¹⁵ fewer of our patients might have indicated a preference to trade if actuarial tables had been used.

Last, our posing the questions in a time trade-off format similar to that used in the McNeil et al¹⁵ study created circumstances incongruous to the natural history of head and neck cancer. Recurrent or persistent laryngeal cancer is not simply a "trade-off" of survival in the 9th or 10th year of survival, but is instead a decrease in the percentage of survivors, typically observed in the first 5 years following treatment. Additionally, although not all laryngeal preservation protocols have demonstrated similar survivals to that of a laryngectomy, some have.⁵ Thus, trading off survival to any extent may not accurately reflect all nonsurgical options. Unfortunately, the two other well-accepted methods to format utility questions (ie, "willingness to pay" and "standard gamble") proved unsuccessful when we attempted them. Willingness to pay questions, which ask the patient how much money he or she is willing to pay to either receive or avoid an outcome (depending on whether it is a benefit or harm), proved offensive to our patients. Standard gamble questions, which pose questions in the form of lotteries, proved too confusing to our patients. This, in fact, raises an even more critical issue: Does a good tool currently exist for utility assessment in the head and neck cancer patient? Future studies must address this issue and work to establish a reliable and valid method for assessing utility in laryngeal cancer patients. This will then empower HCPs to more effectively identify patients who will benefit from laryngeal preservation attempts.

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RECONSTRUCTIVE MICROSURGERY IN HEAD AND NECK CANCER

The First International Symposium on Reconstructive Microsurgery in Head and Neck Cancer will be held in Pavia, Italy, September 11-13, 1997. For more information, contact M. Benazzo, MD, University of Pavia, Policlinico S. Matteo, P.le Golgi 2, 27100 Pavia, Italy; telephone +39 382 528184; fax +39 382 528184; e-mail: orl37@ipv36.unipv.it or mullacc@mbox.vol.it.

GLOTTIC-SUPRAGLOTTIC BARRIER: FACT OR FANTASY?

JOHN A. KIRCHNER, MD

NEW HAVEN, CONNECTICUT

An anatomic structure that might act as a barrier between the glottic and supraglottic areas has never been demonstrated in whole organ serial section studies. Nevertheless, most squamous cell carcinomas arising on the supraglottic mucosa remain confined above the ventricle, and this fact is reflected in the high rate of local control obtained by surgeons performing horizontal supraglottic laryngectomy: 80.6% to 98%. Whole organ sections of laryngectomy specimens suggest that a tumor situated above and below the glottic level may have arrived there not by crossing the ventricle, but by encircling it. In so doing, part of the tumor is visible posterior to the ventricle or on the arytenoid cartilage — a finding that contraindicates conventional supraglottic laryngectomy, with or without limited mobility of the true vocal cord.

KEY WORDS — glottic-supraglottic barrier, supraglottic carcinoma, transglottic carcinoma, ventricle.

Does a barrier exist between the glottic and supraglottic parts of the larynx — a barrier that would prevent the spread of carcinoma from the glottis to the supraglottis and vice versa? This question has been debated since the latter half of the 19th century and was brought into focus by the work of Hajek,¹ which showed that injected fluids did not cross the ventricle, but remained confined to the supraglottic or subglottic area according to the site of injection. In spite of this and subsequent experiments that indicate the presence of intralaryngeal compartments, an anatomic structure that might act as a barrier between the glottic and supraglottic areas has never been demonstrated in whole organ serial section studies.

The most likely candidate at the supraglottic level is the quadrangular membrane because of its proximity to the overlying supraglottic mucosa where such tumors originate. It has not, however, been identified around the advancing edge of a supraglottic tumor, even with elastic tissue stains. The other fibroelastic membrane in the ventricular area is the thyroglottic ligament, but it shows numerous dehiscences in coronal sections, and does not reach the thyroid lamina in all planes.² Nevertheless, it cannot be denied that most squamous cell carcinomas arising on the supraglottic mucosa remain confined above the ventricle, and that this fact is reflected in the high rate of local control obtained by surgeons performing horizontal supraglottic laryngectomy: Bocca et al (80.6%),³ Som (95%),⁴ DeSanto (98%),⁵ Lutz et al (98%),⁶ Herranz-González et al (94.6%),⁷ and others. Som's experience was typical. Of 93 patients followed up from 2 to 17 years, there were 5 local recurrences, 4 of which, while infiltrating the base of the tongue and the pharyngeal wall, left the glottis free of tumor.

Surgical specimens provide further evidence of the oncologic independence of these two laryngeal components. The enormous specimen shown in Fig 1, for example, acquired after the serial sectioning project at Yale had been terminated, defied classification until the ventricle was found. Tumor had not crossed its fundus, the true vocal cords were soft, the floor of the ventricle was free of visible tumor, and the thyroid lamina, examined by the pathologist, showed no invasion in multiple sections. It was probably incurable by any type of surgical procedure, but is shown

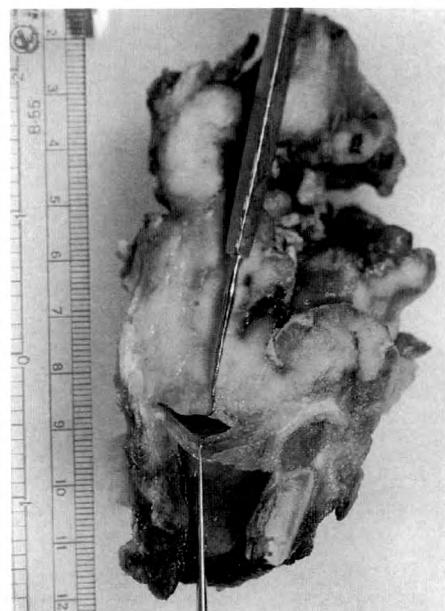


Fig 1. This mass of tumor proved to be supraglottic after true vocal cord and ventricle were identified. Despite extensive invasion into tongue, tumor had not crossed ventricle, and thyroid cartilage was free of tumor in multiple sections.

From the Section of Otolaryngology, Dept of Surgery, Yale University School of Medicine, New Haven, Connecticut.

CORRESPONDENCE — John A. Kirchner, MD, Section of Otolaryngology, Dept of Surgery, Yale University School of Medicine, 333 Cedar St, PO Box 208041, New Haven, CT 06520-8041.

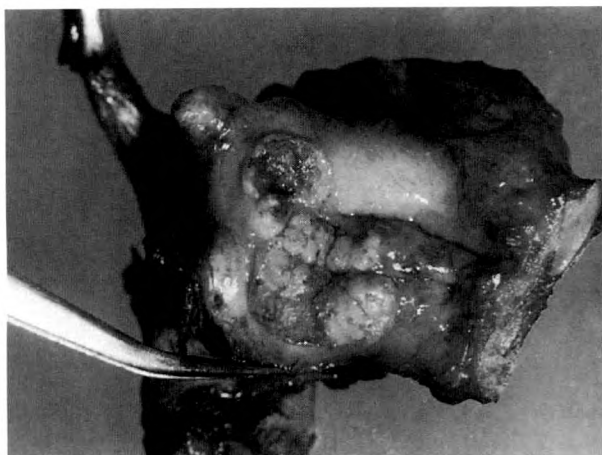


Fig 2. Hemilaryngectomy specimen showing glottic carcinoma extending to arytenoid cartilage. Fundus and roof of ventricle were free of tumor in serial sections. This specimen may represent intermediate stage in development of transglottic carcinoma.

mainly to demonstrate three principles: 1) supraglottic carcinoma spreads mainly upward (cranially); 2) supraglottic cancer large enough to invade the base of the tongue may still spare the ventricle; and 3) supraglottic cancer does not invade the laryngeal framework.⁸

Despite these facts, a small proportion of supraglottic cancers whose main bulk occupies the epiglottic petiole or the false vocal cord may invade the substance of the true cord. How can this statement be reconciled with the principle that carcinoma rarely extends downward across the ventricle? The answer to this apparent dilemma may be available in whole organ sections. Such studies provide strong evidence for a pathway by which supraglottic carcinoma can invade the glottic structure, or by which glottic cancer can invade the supraglottis, without actually "crossing" the ventricle in either case. In a report by Glanz,⁹ for example, the spread of glottic carcinoma was studied in step sections. Tumor is shown in several specimens to ascend posterior to the ventricle by a pathway into the supraglottic component of the larynx. A representative specimen from our practice is shown in Fig 2.

The same portal of invasion is observable in several "supraglottic" tumors in our collection. These were removed at an early enough stage to reveal how supraglottic cancer can invade the glottic musculature without actually crossing the ventricle (Fig 3). By infiltrating the arytenoid area, cancer can surround the posterior end of the ventricle and invade the glottic musculature from behind. In this way the tumor encircles the ventricle from above and below, like the upper and lower parts of a crab's claw. Glanz⁹ states that this is the route by which glottic carcinoma

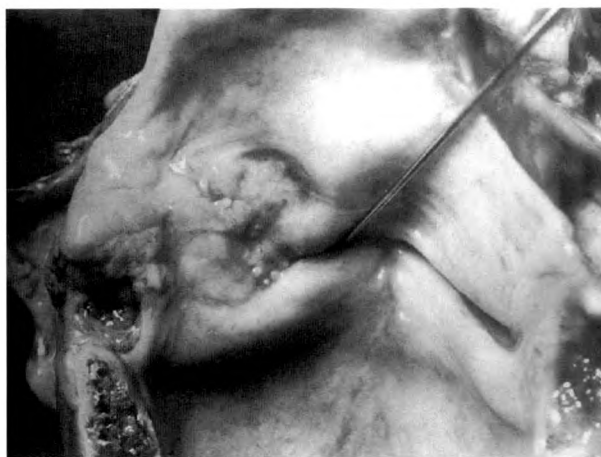


Fig 3. Total laryngectomy specimen. Carcinoma involves false vocal cord and has invaded true cord submucosally, with fixation. Patient had been treated unsuccessfully with irradiation. Probe is inserted into anterior portion of ventricle, still largely uninvaded.

can envelop the ventricle and become "multiregional" or "transglottic," the latter term having been popularized in the American literature by McGavran et al.¹⁰ In its anterior progress, cancer may obliterate the ventricle, leaving the observer with no evidence that would indicate whether the ventricle had been crossed or merely encircled in its earlier development (Fig 4).

Of what practical value are these observations, one might ask, assuming that they are valid? They are useful because they provide contraindications to supraglottic laryngectomy, on the basis of either limited vocal cord mobility, the presence of visible tumor posterior to the ventricle in the arytenoid area, or visible tumor at the anterior commissure extending from the petiole to the true vocal cords. In Figure 3 of

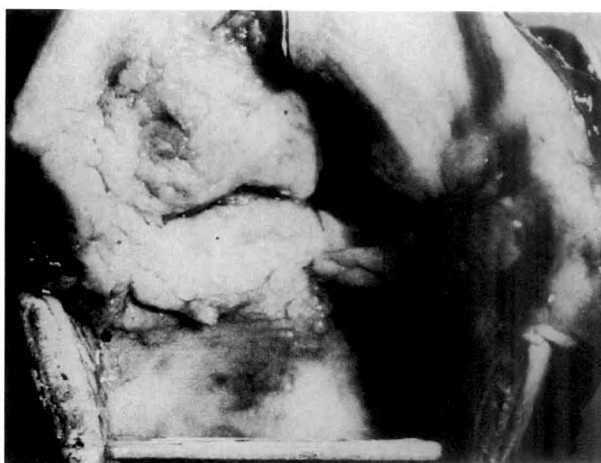


Fig 4. Total laryngectomy specimen opened through cricoid plate. Ventricle, seen here as horizontal slit in tumor, is completely surrounded by carcinoma. "Transglottic" in this case seems less accurate term than "multiregional," since tumor appears to have encircled ventricle rather than to have crossed it.

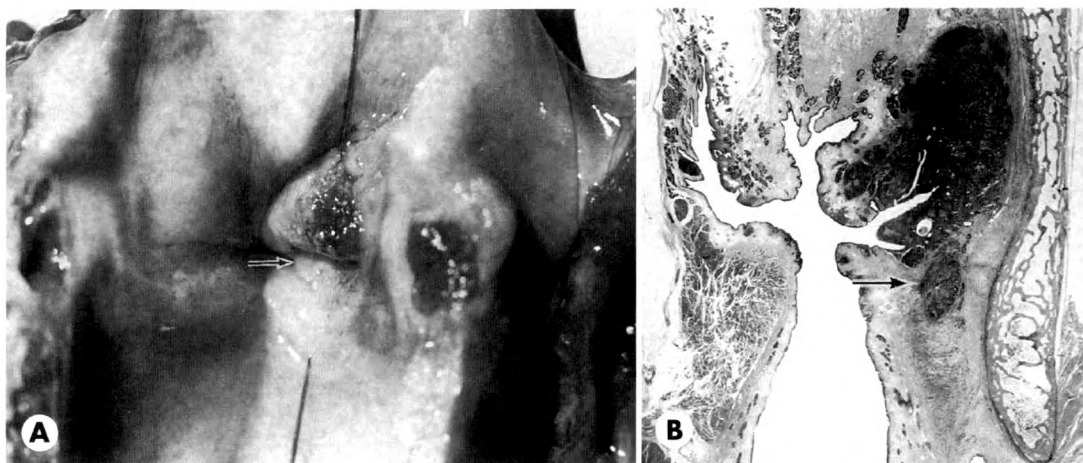


Fig 5. A) Total laryngectomy specimen showing squamous cell carcinoma deep within ventricle (arrow). It appears to have arisen in ventricle, since bulk of tumor anterior and posterior to this section remained entirely submucosal. B) Coronal section of specimen shown in A. Carcinoma has invaded thyroarytenoid muscle (arrow) by outflanking or penetrating thyroglottic membrane along floor of ventricle. It has invaded supraglottis by undermining quadrangular membrane. This behavior is typical of cancer originating deep within ventricle.

Tucker's report,¹¹ for example, the "supraglottic" lesion, removed by total laryngectomy, extends downward deep to the surface of the true cord (section 572) and invades the arytenoid area in section 960. Tumor on the arytenoid would have been obvious to the surgeon, had horizontal supraglottic laryngectomy been considered.

These observations do not mean that cancer found above and below the ventricle or anterior commissure has followed this pattern of invasion in every case. In the author's collection of 91 laryngectomy specimens classified as transglottic and studied by whole organ sections, it was often impossible to determine the point of origin. Available evidence suggests that a tumor situated above and below the glottic level may have arrived there by one of the following three routes.

1. Glottic cancer spreading along the floor of the ventricle may outflank the thyroglottic ligament and invade the glottic musculature. As it advances, it invades the supraglottic larynx, undermining and displacing the quadrangular membrane (Fig 5). The tumor in Figure 16 in Baclesse's report¹² probably followed the same route of invasion.

2. Cancer at the epiglottic petiole anterior to the crease between the false vocal cord and the base of the epiglottis is normally confined above the glottis by the anterior commissure tendon.¹³ Much less often, cancer crosses this tendon and invades the anterior parts of the true vocal cords. This type of lesion is usually ulcerated and is associated with a high incidence of invasion into the adjacent laryngeal framework. Despite its aggressiveness, it may be associated with normal mobility in the more posterior parts

of the true vocal cords. It is difficult to assign this type of lesion to either the glottic or supraglottic category.¹⁴

3. Tumor arising on the supraglottic mucosa may spread downward posterior to the ventricle and from this position invade the glottic region. This type of spread is signalled by tumor visible on the arytenoid or behind the ventricle, with or without limited mobility of the true vocal cord, and contraindicates conventional supraglottic laryngectomy. In each of these patterns of spread, the tumor is classified "transglottic."

DISCUSSION

Much of the confusion about the behavior of supraglottic carcinoma and about a possible barrier between the glottic and supraglottic components of the larynx results from terminology. Classifying those supraglottic tumors that spread below the ventricle as "supraglottic T3" undoubtedly accounts for the disproportionately high incidence of supraglottic carcinoma in some parts of the world (Finland, Chile, northeastern China, etc). Many of these tumors would have been classified "transglottic" or "multiregional" in other clinics and excluded from the supraglottic category in analysis of results of treatment.

A detailed discussion of the present staging system is beyond the scope of this report, but a comment on the term "transglottic" may clarify the reasons for its use in those lesions whose bulk is predominantly supraglottic.

1. It describes where the tumor *is*, rather than a supposition as to *where it began*.¹⁵ Large transglottic lesions are difficult to assign to one of the conven-

tional categories.

2. "Transglottic" differentiates between those T3 supraglottic tumors that invade the vocal cord area and those that qualify for the T3 category merely because they invade the preepiglottic space. The first group—above and below the ventricle—invade the laryngeal framework in a high proportion of cases, and cannot be treated by supraglottic laryngectomy. The second group, the genuine supraglottic cases, without tumor visible at the anterior commissure or posterior to the ventricle, do not invade the laryngeal framework,⁸ and are highly curable by supraglottic laryngectomy. Grouping both types as T3 supraglottic is surgically, and statistically, unsound.

The immediate question is whether such tumors invade both parts of the larynx by crossing the ventricle. Gross and histologic examination of surgical specimens removed by total laryngectomy suggests that they do not. Except for the anterior commissure lesion described above, the most likely pathway is posterior to the ventricle, with the tumor encircling it from above and below. This proposal cannot be stated with absolute certainty, since the developing stages leading up to a total laryngectomy are never available for study in serial sections. Even then, whole organ sections may be misleading if they are not displayed at multiple planes, and this is difficult to demonstrate in the usual report because of space limitations. In the classic report by Olofsson and van Nostrand,¹⁶ on the other hand, some of the 139 serially sectioned laryngectomy specimens were displayed in planes from the front of the larynx to the back, and these demon-

strate the validity of the criteria for selecting cases suitable for supraglottic laryngectomy. Their Figure 15, for example, demonstrates a supraglottic lesion spreading into the paraglottic area below the ventricle, but sections made through the retroventricular area and arytenoid cartilage reveal cancer that would have been obvious to the surgeon and alerted him or her that supraglottic laryngectomy was contraindicated. Similar lesions, identifiable by tumor posterior to the ventricle, are included in the reports of Tucker¹¹ and Glanz.⁹

The practical aspect of the barrier question is whether an individual supraglottic tumor can be safely resected by supraglottic laryngectomy. The selection criteria published by Bocca et al³ and Som⁴ provide assurance that any individual supraglottic tumor has not spread below the ventricle, and that supraglottic laryngectomy in such cases results in complete removal of the laryngeal tumor. In pointing out that Bocca supports his "barrier" concept on the basis of gross examination of 150 supraglottic carcinomas without performing histologic analysis, Weinstein et al¹⁷ fail to explain how Bocca and other surgeons have achieved local control rates of 80.6% to 98% by supraglottic laryngectomy. Such success casts considerable doubt on the proposal that unrecognized cancer often exists below the ventricle or anterior commissure in supraglottic lesions properly evaluated for conservation surgery. Examination of coronal sections in multiple planes strongly suggests that the ventricle does not act as a "funnel," as these authors propose, but more like a chasm, seldom crossed, though often encircled and obliterated.

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ROLE OF FLEXIBLE LARYNGOSCOPY IN EVALUATING ASPIRATION

GLENN M. KAYE, MD
NEWARK, NEW JERSEY

RICHARD D. ZOROWITZ, MD
PHILADELPHIA, PENNSYLVANIA

SOLY BAREDES, MD
NEWARK, NEW JERSEY

Flexible fiberoptic laryngoscopy is used to evaluate dysphagia, but its clinical utility has not been compared to that of the videofluorographic swallowing study (VFSS). This study correlates parameters of both procedures and identifies laryngoscopic predictors of aspiration in 105 patients. Presence of aspiration, pharyngeal residue, laryngeal sensation, vocal cord mobility, and glottic closure during flexible laryngoscopy (FL), and gag reflex were correlated with aspiration during the VFSS. An algorithm for laryngoscopically detecting aspiration was synthesized. Aspiration ($p = .004$) and pharyngeal residue ($p < .00001$) were highly correlated between the two studies. Aspiration during the VFSS was correlated with pharyngeal residue ($p < .00001$) and laryngeal sensation ($p = .027$) during FL, but not glottic closure ($p = .169$) nor vocal cord mobility ($p = .056$). Patients with a normal gag reflex and without aspiration or pharyngeal residue during FL had a 2.94% risk of aspiration during the VFSS. Flexible laryngoscopy can be used as a relatively safe, portable screening test for aspiration, but cannot always replace the VFSS to identify the presence or cause of aspiration.

KEY WORDS — aspiration, deglutition disorders, fluoroscopy, laryngoscopy, video recording.

INTRODUCTION

Dysphagia is a common problem associated with neuromuscular diseases, head and neck neoplasm, and even psychological disorders. The evaluation of swallowing function is essential not only to identify the nature of the swallowing disorder(s), but also to prescribe appropriate interventions. The current "gold standard" for evaluation of dysphagia is the modified barium swallow study, or videofluorographic swallowing study (VFSS). The VFSS provides real-time visualization of the oral cavity, oropharynx, laryngopharynx, and esophagus by means of measured or functional amounts of barium contrast in liquid, paste, and solid forms. The study may be viewed in both anteroposterior and lateral views and measures parameters such as the duration of bolus transit, the degree of pooling, and the timing and severity of aspiration. With this information, clinicians can assess a patient's success in swallowing food of different amounts and consistencies, with and without compensatory strategies.

However, the VFSS has several drawbacks. It requires the facilities of a radiology suite, including fluoroscope, monitor, and personnel. The patient must be exposed to radiation to show the anatomy of the swallowing mechanism. The patient also must be transported to the suite, be positioned for the study,

and cooperate with the examination. Although the VFSS is excellent in characterizing overall swallowing ability and defining functional deficits and degree of aspiration, it does not always identify subtle abnormalities in pharyngeal, hypopharyngeal, and laryngeal anatomy or sensation.

The flexible fiberoptic nasolaryngoscope provides a relatively safe means of evaluating dysphagia. It provides direct visualization of the pharynx during swallowing and allows the clinician to assess anatomic and physiologic deficits of the palate, pharynx, and larynx, as well as sensation of the upper aerodigestive tract, pooling of secretions, and the patient's ability to swallow test substances of various consistencies with appropriate compensatory strategies as needed. It can be speedily performed, even at the bedside, with minimal positioning of the patient and involves no exposure to radiation or other significant deleterious consequences. It can be performed repeatedly over time to assess changes in a patient's swallowing function, without the inconvenience of waiting for a VFSS to be scheduled.

Flexible fiberoptic laryngoscopy has been studied in the evaluation of swallowing function in dysphagic patients.¹⁻⁹ Only one article has calculated the sensitivity, specificity, and predictive value of similar parameters between the VFSS and flexible laryn-

From the Department of Physical Medicine and Rehabilitation (Kaye, Zorowitz) and the Section of Otolaryngology-Head and Neck Surgery (Baredes), University of Medicine and Dentistry of New Jersey/New Jersey Medical School, Newark, and the Center for Swallowing Disorders (Zorowitz), Kessler Institute for Rehabilitation, East Orange, New Jersey.

CORRESPONDENCE — Richard D. Zorowitz, MD, Dept of Rehabilitation Medicine, University of Pennsylvania Medical Center, 5 West Gates, 3400 Spruce St, Philadelphia, PA 19104-4283.

TABLE 1. PARAMETERS RECORDED

VFSS	FL	Physical Examination
Aspiration	Aspiration	Gag reflex
Pooling	Pooling	
	Velopharyngeal insufficiency	
	Vocal cord mobility	
	Glottic closure with breath-holding	
	Laryngeal sensation	

VFSS — videofluorographic swallowing study, FL — flexible laryngoscopy.

gосcopy (FL) in a small sample of patients.⁶ No studies to date have demonstrated the actual clinical utility of FL as compared to the VFSS in evaluating the dysphagic patient.

The objectives of this study are to evaluate the use of FL in a larger sample population and to determine the clinical usefulness of FL compared to the VFSS, specifically for the evaluation of aspiration. The hypothesis to be tested is that FL may be used exclusively for the evaluation of specific elements of swallowing function, including aspiration. If this is true, then FL can replace the VFSS for evaluating certain swallowing disorders, since performing both studies in all dysphagic patients may be redundant.

METHODS

The charts of all patients examined at the Center for Swallowing Disorders, Kessler Institute for Rehabilitation, East Orange, NJ, between February 1, 1990, and June 30, 1995, were reviewed. During their examinations, patients underwent both a VFSS and FL for evaluation of dysphagia. All patients were first examined by a physiatrist (R.D.Z.) to assess the function of cranial nerves II through XII bilaterally. The gag reflex was tested by holding a cotton swab against the posterior oropharyngeal wall for up to 10 seconds or until a gag was elicited.

The FL was performed by an otolaryngologist (S.B.) using an Olympus model ENF Type P3 flexible fiberoptic nasolaryngoscope fitted with a Javelin model JE 3262 video camera. During the procedure, velopharyngeal function, vocal cord mobility, pooling of secretions in the valleculae or pyriform sinuses, and glottic closure with breath-holding were observed. The patients then swallowed boluses of water dyed with blue food coloring in amounts of 1, 3, or 5 mL. During the swallowing trials, the presence or absence of aspiration, and pharyngeal residue of the bolus in the valleculae or pyriform sinuses was observed. Finally, an estimate of laryngeal sensation was made by observing the gag response to touching

TABLE 2. DISTRIBUTION OF DIAGNOSES

Diagnosis	Patients	
	No.	%
Stroke	26	24.8
Primary dysphagia	22	20.9
Head and neck surgery	19	18.1
Traumatic brain injury, anoxia, or craniotomy	19	18.1
Other*	19	18.1

*Includes cervical myelopathy, chronic fatigue syndrome, Hashimoto's thyroiditis, motor neuron disease, multiple sclerosis, parkinsonism, poliomyelitis, and tic douloureux.

the laryngoscope against the epiglottis or laryngeal vestibule.

The VFSS was performed with a Picker fluoroscope. The studies were performed by a speech-language pathologist under the supervision of the physiatrist. The patients were given thin liquid in boluses of 1, 3, 5, or 10 mL, and then were given thin liquid by cup. In some studies, thick liquid was given prior to thin liquid. If a patient significantly aspirated thick liquid, then a thin liquid trial was not performed, since it is assumed that a patient who cannot manage thick liquid would not be able to manage the more difficult thin liquid.

The parameters recorded from each study are listed in Table 1. Velopharyngeal sufficiency, glottic closure with breath-holding, and laryngeal sensation were recorded as normal or abnormal, present or absent. Pharyngeal residue of the bolus was considered to be present if found in either the valleculae or pyriform sinuses. Vocal cord mobility was recorded as normal, abnormal compensated, or abnormal uncompensated. The gag reflex on physical examination was recorded as normal, impaired, or absent.

The data were analyzed with SPSS for Windows version 6.1 (SPSS, Inc, Chicago, Ill, 1994). Correlations between variables were determined by χ^2 analysis with either the Fisher's exact two-tailed test or the Pearson correlation coefficient, depending on the size of the sample cells. An algorithm incorporating the most significant predictors for aspiration was generated with SPSS CHAID (Chi-squared Automatic Interaction Detector),¹⁰ which divides a population into two or more distinct groups based on categories of the "best" predictors of a dependent variable.

RESULTS

A total of 105 patients completed the full evaluation. The median age of the patients was 61 years (range 19 to 88 years). The distribution of diagnoses associated with swallowing problems is listed in

TABLE 3. FL VERSUS VFSS FOR DETECTING ASPIRATION AND PHARYNGEAL RESIDUE

	<i>Sensitivity</i>	<i>Specificity</i>	<i>PPV</i>	<i>NPV</i>	<i>Correlation Significance</i>	<i>Relative Risk (95% CI)</i>
Aspiration	21.9%	97.2%	77.8%	73.4%	p = .00364	2.92 (1.80 - 4.75)
Pharyngeal residue	90.9%	85.5%	75.0%	95.2%	p < .00010	15.5 (5.07 - 47.4)

FL — flexible laryngoscopy, VFSS — videofluorographic swallowing study, PPV — positive predictive value, NPV — negative predictive value, CI — confidence interval.

Table 2.

Physical Examination. During the physical examination, the gag reflex was absent in 14 of 98 patients (14.3%), impaired in 42 of 98 (42.9%), and normal in 42 of 98 (42.9%).

Flexible Laryngoscopy. During FL, aspiration occurred in 9 of 103 patients (8.7%). Vocal cord mobility was normal in 68 of 101 (67.3%), abnormal compensated in 16 of 101 (15.8%), and abnormal uncompensated in 17 of 101 (16.8%). Pooling of bolus residua or secretions was observed in the valleculae or pyriform sinuses in 42 of 104 (40.4%). Laryngeal sensation was abnormal in 28 of 103 (27.2%). Glottic closure with breath-holding was incomplete in 12 of 49 (24.5%). Velopharyngeal insufficiency was present in 25 of 100 (25.0%).

Videofluorographic Swallowing Study. During the VFSS, aspiration occurred in 33 of 105 (31.4%) patients. Pooling of bolus residua in the valleculae or pyriform sinuses occurred in 33 of 103 (32.0%).

FL Versus VFSS: Similar Parameters. Parameters observed during both FL and the VFSS are compared in Table 3. Observations of aspiration and pharyngeal residue were highly correlated between the two diagnostic studies. The relative risk of each of these abnormalities occurring during the VFSS was significant if the corresponding aberrance was present during FL.

Predictors of Aspiration During VFSS. All variables recorded during physical examination and FL were compared with the presence of aspiration during the VFSS, as shown in Table 4. All parameters except glottic closure during breath-holding and vo-

cal cord mobility were significantly correlated with aspiration during the VFSS. Aspiration and pharyngeal residue during FL were both strong risk factors for aspiration during the VFSS. In contrast, abnormal pharyngeal sensation and abnormal gag reflex were weak risk factors for aspiration during the VFSS.

Algorithm for Prediction of Aspiration During VFSS: CHAID Analysis. The Figure¹⁰ demonstrates that aspiration during FL, pharyngeal residue during FL, and abnormal gag reflex on physical examination were the most significant risk factors for aspiration during the VFSS. On CHAID analysis, a 27.08% risk of aspiration during a VFSS exists if aspiration is not observed during FL. In addition, if no pharyngeal residue is observed, then the probability of aspiration during a VFSS drops to 11.86%. If the first two conditions are present and the patient has a normal gag reflex, the chance of aspiration on a VFSS dropped to only 2.94%. In all other cases, the risk of aspiration is still significant, such that a VFSS should be performed to confirm the presence or absence of aspiration.

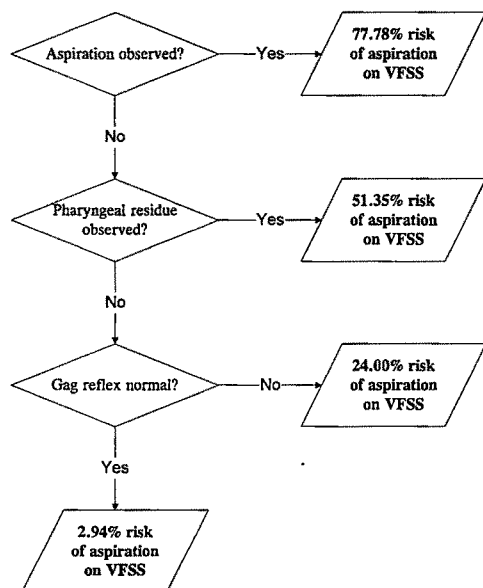
DISCUSSION

Currently, the VFSS is the standard procedure used to evaluate patients with dysphagia, and FL is not often utilized as a primary means of detecting abnormalities in swallowing function. However, FL would be preferable to the VFSS as a screening test in at least some cases, as it decreases radiation exposure and facilitates the evaluation of patients who cannot tolerate or be transported to the VFSS. Deglutition in patients with an evolving disease process could be followed much more closely, since FL can be done at the bedside and at a moment's notice,

TABLE 4. FINDINGS ON FL AND PHYSICAL EXAMINATION FOR PREDICTING ASPIRATION ON VFSS

	<i>Sensitivity</i>	<i>Specificity</i>	<i>PPV</i>	<i>NPV</i>	<i>Correlation Significance</i>	<i>Relative Risk (95% CI)</i>
Aspiration	21.9%	97.2%	77.8%	73.4%	p = .00364	2.92 (1.80 - 4.75)
Pharyngeal residue	72.2%	74.6%	57.1%	85.4%	p < .00001	3.94 (2.04 - 7.60)
Gag reflex	73.3%	50.0%	39.3%	81.0%	p = .03150	2.06 (1.02 - 4.17)
Glottic closure	37.5%	81.8%	50.0%	73.0%	p = .16900	1.85 (0.85 - 4.02)
Pharyngeal sensation	41.9%	79.2%	46.4%	76.0%	p = .02720	1.93 (1.10 - 3.41)
Vocal cord mobility	27.3%	88.2%	52.9%	71.4%	p = .05070	1.85 (1.06 - 3.25)

FL — flexible laryngoscopy, VFSS — videofluorographic swallowing study, PPV — positive predictive value, NPV — negative predictive value, CI — confidence interval.



CHAID analysis¹⁰ tree. Relates observations made during flexible laryngoscopy and physical examination to risk of aspiration on videofluorographic swallowing study (VFSS).

while a VFSS usually needs to be scheduled in advance.

The only other comparison between the two diagnostic procedures is a study of 21 subjects with a variety of primary diagnoses.⁶ In that study, sensitivity, specificity, positive predictive value, and negative predictive value were calculated with respect to four parameters: premature spillage, laryngeal penetration, tracheal aspiration, and pharyngeal residue. Flexible laryngoscopy was greater than 90% sensitive for detecting pharyngeal residue and laryngeal penetration. Specificity was greater than 90% only for tracheal aspiration. Flexible laryngoscopy was 100% accurate for negatively predicting laryngeal penetration on the VFSS, but was less than 90% accurate in positively predicting the occurrence of any of the variables during the VFSS.

While the earlier study presents some important descriptive data, some significant differences are observed in the sensitivities, specificities, positive predictive values, and negative predictive values in detecting aspiration and pharyngeal residue between the two studies (Table 5⁶). These differences may be explained in part by the larger sample size of the present study. In addition, differences between interpretations of "pharyngeal residue" may exist, since the earlier study does not define how the presence of pharyngeal residue is determined. The present study also provides correlative and relative risk statistics relating similar parameters between the procedures and factors predictive of aspiration. Also, the earlier study investigates the ability of FL to detect several

TABLE 5. COMPARISON OF LANGMORE ET AL⁶ STUDY WITH PRESENT STUDY IN ASSESSING FL VERSUS VFSS FOR DETECTING ASPIRATION AND PHARYNGEAL RESIDUE

	Sensitivity	Specificity	PPV	NPV
Aspiration				
Langmore et al ⁶	88.0%	92.0%	88.0%	92.0%
Present study	21.9%	97.2%	77.8%	73.4%
Pharyngeal residue				
Langmore et al ⁶	93.0%	50.0%	82.0%	75.0%
Present study	90.9%	85.5%	75.0%	95.2%

FL — flexible laryngoscopy, VFSS — videofluorographic swallowing study, PPV — positive predictive value, NPV — negative predictive value.

factors without demonstrating their clinical outcome, while the current study focuses on detection of the most important clinical outcome: aspiration.

It is interesting that vocal cord closure, which is considered by some to be the most critical factor for prevention of aspiration,⁹ was not correlated with aspiration on the VFSS. Furthermore, the gag reflex, which itself is not believed to aid in swallowing nor protect from aspiration,⁹ was correlated with aspiration. While the gag reflex may be absent in patients with otherwise normal deglutition, the latter finding probably was observed because an impaired swallowing reflex usually does not exist in isolation, but is associated with other alterations of swallowing function.^{11,12}

Some discrepancies of observation were present in the study. Pharyngeal residue was observed in more patients during FL (42) than during the VFSS (33). This is because clinicians can see pooling of secretions or the bolus by FL, but on the VFSS can only visualize those contents impregnated with barium. Likewise, 2 patients with observed aspiration during FL did not aspirate during the VFSS. This is because a patient may not aspirate consistently during each and every swallow, and the tests are not performed concurrently. Each test is essentially a snapshot in time, and may not accurately represent the overall clinical situation for a given patient. This phenomenon also may explain the low sensitivity attributed to FL in detecting aspiration.

In conclusion, this study demonstrates that FL can be used as a screening tool for the evaluation of dysphagia. However, it cannot always replace the VFSS to identify the presence and cause of aspiration. With a detailed physical examination, FL can rule out aspiration with up to 97% certainty. When aspiration is suspected in a patient with dysphagia, however, the VFSS still remains the most valid and reliable procedure to aid in diagnosing swallowing disorders and formulating a plan for dysphagia therapy.

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IMAGING CASE STUDY OF THE MONTH KNIFE BLADE AS A FACIAL FOREIGN BODY

PHILLIP A. GARDNER, MD

PAUL RIGHI, MD

PAUL B. SHAHBAHRAMI, MD

INDIANAPOLIS, INDIANA

INTRODUCTION

A retained knife blade as the cause of a foreign body in the face is quite rare. Patients presenting to the emergency department (ED) with a knife wound to the face require at least superficial exploration of the wound to rule out a foreign body and to assess the extent of the injury. Routine radiographic screening of all such patients is not performed unless there is suspicion of a foreign body or neurovascular injury. In this report, we describe a rare case of a large knife blade retained in a facial wound that was not discovered on initial physical examination in the ED. The diagnosis was made 3 weeks later during a subsequent ED visit prompted by trismus. Plain films of the face at that time revealed the knife blade foreign body. In our report, we emphasize the need for a thorough history regarding the weapon, accurate wound exploration, and routine plain films of the face unless the weapon used to inflict the facial stab injuries is recovered and known to be intact.

In addition, an arteriogram is needed to rule out vascular injury if a facial foreign body is located near the carotid arterial system. Wound exploration under general anesthesia to remove the foreign body may require special anesthetic techniques, as in our case, where severe trismus prompted fiber-optic endotracheal intubation.

CASE REPORT

A 27-year-old man was evaluated in the ED of a local referral hospital after suffering a stab wound to the left side of his face. The weapon reportedly used was a steak knife. The initial ED report did not mention if the knife was ever recovered. On examination, no active bleeding from the two facial wounds was noted and there was no history of pulsatile bleeding. Hemodynamically, the patient was stable. The facial wounds were approximately 0.5 cm and 1.0 cm in length and located just inferior to the left

malar prominence. On intraoral examination, a puncture wound in the left upper buccal mucosa was noted. Seventh cranial nerve function was preserved and the parotid duct and parotid gland were intact. The wounds were examined and then closed by the evaluating ED physician. A tetanus toxoid was also administered. The patient was given a follow-up appointment in 4 days for suture removal.

Three days later the patient returned to the same ED complaining of pain and swelling around the wound. An oral surgery consultation was obtained. The patient was subsequently placed on a 10-day course of clindamycin for a presumed facial cellulitis and given a follow-up appointment with the oral surgery department, which he failed to keep.

Approximately 3 weeks later, the patient again returned to the ED complaining of significant pain and difficulty opening his mouth. Minimal swelling around the incision was noted on examination; however, a hard mass was present on palpation beneath the left cheek scar. The patient had trismus to the extent that his interincisor opening was only two finger-breadths wide. Posteroanterior and lateral plain films of the face revealed a 9.0-cm radiopaque serrated foreign body that resembled a knife blade (Fig 1). The foreign body extended posterolaterally from the soft tissues beneath the left malar eminence so that it rested adjacent to the left side of the tongue base in the deep neck soft tissue planes. The pharynx was not penetrated by the foreign body on radiographic or flexible endoscopic examination. Otolaryngological consultation was obtained and an angiogram was performed due to the proximity of the foreign body to the carotid arterial system. The angiogram was negative for vascular injury (Fig 2).

The patient was then taken to the operating room for removal of the presumed knife blade. The patient's trismus and the desire not to manipulate the knife

From the Department of Otolaryngology-Head and Neck Surgery, Indiana University School of Medicine, Indianapolis, Indiana.

CORRESPONDENCE—Paul Righi, MD, Dept of Otolaryngology-Head and Neck Surgery, Indiana University Medical Center, 702 Barnhill Dr 0860, Indianapolis, IN 46202-5230.

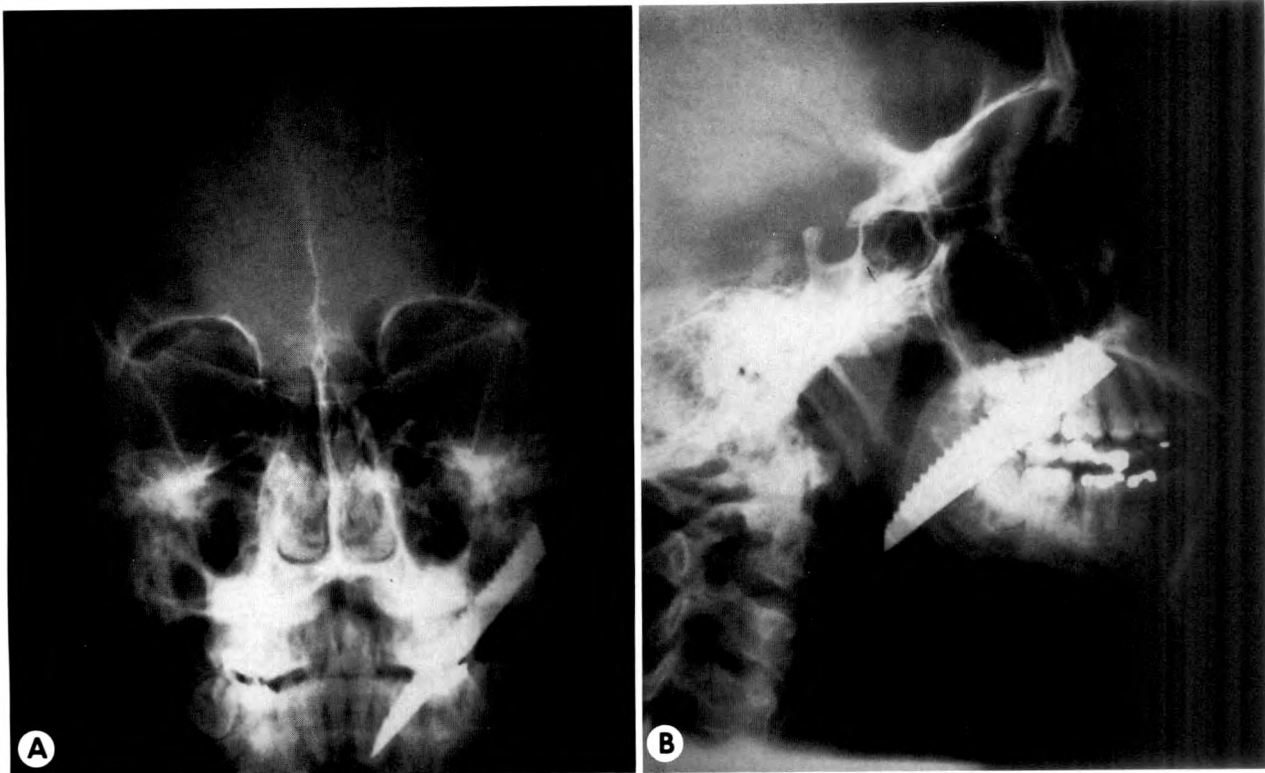


Fig 1. Plain films of skull. Radiopaque object is seen inferior to temporal process of zygomatic bone, coursing posterior, inferior, and medial, ending in posterior pharynx. No fractures or penetration of sinuses are noted. A) Anteroposterior. B) Lateral.

blade with a laryngoscope prompted fiber-optic endotracheal intubation. Under general anesthesia, the cheek scar was incised and the buttress of the blade was found superficially. A 9.0-cm knife blade was carefully withdrawn along its line of entry without incident. Direct laryngoscopy revealed no pharyngeal penetration. The wound was thoroughly irrigated and then drained for 24 hours with a 1/4-in

Penrose drain. Intravenous antibiotics were given initially, followed by a 10-day course of oral antibiotics. The patient was discharged the day after surgery. He failed to keep follow-up appointments.

DISCUSSION

While lacerations of the face are frequently encountered in the ED, foreign bodies do not often result from these injuries. It is therefore useful to establish guidelines for determining when facial radiographs are necessary to rule out a foreign body in patients with facial lacerations. Most of the published experience with this type of injury is from South Africa, where stab wounds to the face are not uncommon.¹⁻⁴ Grobbelaar and Knottenbelt⁵ reviewed a series of 11 patients with retained knife blades in the face, and found that 2 patients had negative findings on clinical examination despite radiographic evidence of a foreign body. They concluded that radiographic survey was required for all but the most superficial stab wounds of the face.⁵

In our case the clinical examination was negative on two evaluations, so no radiographs were obtained until the patient presented with significant trismus. Radiographic survey early on would have been prudent given the following considerations: the facial wound was deep, the knife had not been recovered,

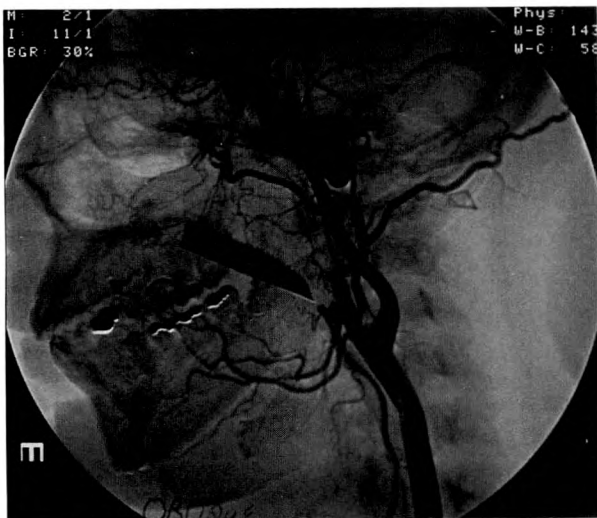


Fig 2. Angiogram of left common carotid artery. Knife blade enters between peripheral branches of left internal maxillary artery superiorly and facial artery inferiorly. No vascular injury is seen.

and the potential foreign body was metallic and thus radiopaque.

Based on our experience with this case and a review of literature, the following recommendations are offered regarding stab wounds to the face from a knife or similar weapon. First, a careful history from the patient, paramedics, or witnesses is crucial to determine if the knife blade was intact after the stabbing. Second, a complete head and neck physical examination should include an assessment of the extent and depth of the stab wound, as well as a complete evaluation of relevant neurovascular structures. Injury or compromise of the airway is given priority, as well as possible injury to the carotid arterial system. In addition, all cranial nerves, including the facial nerve, should be tested and the integrity of the parotid duct assessed.^{6,7} Pharyngeal penetration by the foreign body is another important consideration and can be determined at endoscopy. Gastrografin swallow can also be used for this purpose, but may miss a small mucosal tear. If there is any doubt that the knife was intact afterward, or if the physical examination reveals a deep laceration (into soft tissue or deeper), then appropriate screening radiographs should be obtained.⁸⁻¹⁰ In this manner, the possibility of a retained knife blade in the face is minimized.

In addition, trismus is a worrisome symptom in a patient who has been stabbed in the face. Trismus is due to a combination of pain and muscle spasm, and in the scenario of penetrating trauma to the face, implies deep injury to the muscles of mastication.¹¹ Trismus can result from a foreign body embedded in the mastication muscles, as in our case, or can result from a fracture of the zygomatic arch or a mandibular fracture.^{12,13} The cause of trismus must be elucidated in this setting and should prompt a radiographic evaluation.

The role of preoperative angiographic studies for penetrating trauma in close proximity to major ves-

sels in the head and neck is well covered in the literature and will not be covered in detail here. However, it is important to stress that penetrating trauma to the face from a knife blade in close proximity to the carotid system requires an angiogram prior to surgical removal of the blade to rule out vascular compromise.^{6,14} Although transection of the common carotid artery is invariably fatal, less severe injuries to the internal carotid artery or branch of the external carotid artery are compatible with survival if diagnosed and treated expediently. Furthermore, delayed pseudoaneurysm of the intracranial internal carotid artery has been known to occur following penetrating facial injury, later presenting with fatal epistaxis.^{3,15,16} Repeat angiography is requisite 2 weeks post-trauma in cases where compromise of the vessels has been demonstrated to rule out pseudoaneurysm formation.²

Finally, clear communication with the anesthesiologist prior to surgery is crucial in any head and neck trauma case, but particularly in a case like ours, in which the retained foreign body produced significant trismus requiring judicious management of the airway. In our case, the airway was secured by fiberoptic endotracheal intubation according to the recommendations set forth by Benumof.¹⁷ Fiber-optic intubation has proven to be an effective alternative to tracheostomy in some cases in which traditional laryngoscopic intubation of the patient cannot be accomplished.¹⁸⁻²¹

SUMMARY

This case demonstrates the unpredictability of foreign bodies in the face. The retained knife blade eluded detection on two separate examinations. The essential components to making a correct diagnosis of a foreign body following a stabbing to the face include a thorough review of the mechanism of injury, a complete head and neck examination, a high index of suspicion, and plain radiographs of the face.

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AURICULAR AND MIDDLE EAR MALFORMATIONS, EAR DEFECTS AND THEIR RECONSTRUCTIONS

The Third International Symposium on Auricular and Middle Ear Malformations, Ear Defects and Their Reconstructions will be held in Lubeck, Germany, September 6-9, 1997. For more information, contact Dr H. Weerda or Dr R. Siegert, Department for Otorhinolaryngology and Plastic Head and Neck Surgery, Medizinische Universität zu Lubeck, Ratzeburger Allee 160, D-23538 Lubeck, Germany; telephone (0) 451 500 3189; fax (0) 451 500 4192.

IMAGING CASE STUDY OF THE MONTH

OSTEOMA OF THE MIDDLE EAR

EARL H. HARLEY, MD
WASHINGTON, DC

ROBERT G. BERKOWITZ, FRACS
MELBOURNE, AUSTRALIA

INTRODUCTION

Osteomas are rare in the temporal bone. Most commonly, they occur in the external auditory canal.¹ A review by Denia et al² was notable for the absence of these lesions in the middle ear. In their report on osteoma of the temporal bone, none of 53 patients had middle ear involvement.² A recent paper noted that only nine cases with middle ear involvement have been previously described.³ This report adds another case to the body of literature.

CASE REPORT

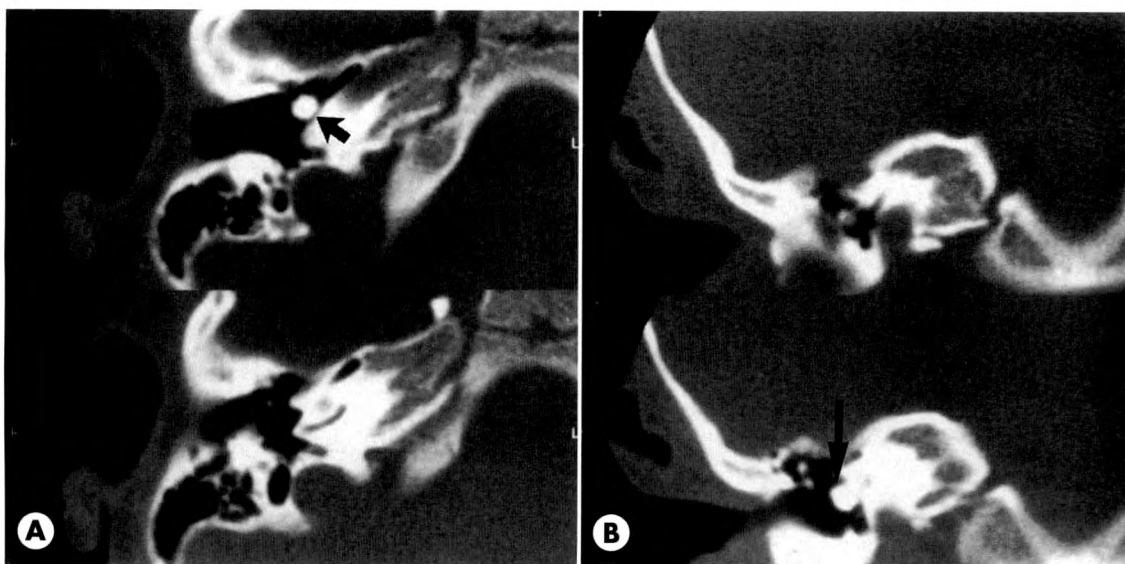
A 5-year-old girl presented to her primary care physician with a history of recurrent otalgia and recurrent acute otitis media. Examination of the ears was remarkable for what appeared to be a cyst behind the right tympanic membrane. The audiologic findings were significant for bilateral mild low-frequency conductive hearing losses. Impedance testing revealed bilateral negative pressure readings.

Otolaryngological evaluation confirmed the presence of what appeared to be a small cystic mass behind an intact right tympanic membrane. It was elected not to obtain any imaging study, because the lesion was deemed to be a small, well-circumscribed congenital cholesteatoma. A transmeatal tympanotomy was undertaken to remove the lesion. At surgery, a dense whitish mass was encountered on the medial wall of the mesotympanum. An incisional biopsy was attempted, but complete removal of the mass was deemed inadvisable. Histologic review of this lesion yielded a diagnosis of osteoma.

Postoperatively, computed tomography of the petrous bone was obtained. A 3- to 5-mm rounded structure of uniform density similar to that of the adjacent bone projected into the middle ear cavity (see Figure). The bony structures of the inner ear were normal.

DISCUSSION

Osteomas are rare in the temporal bone. The middle



Computed tomograms of right temporal bone. Arrows — osteoma in middle ear. A) Axial view. B) Coronal view.

From the Department of Otolaryngology, The Royal Children's Hospital, Melbourne, Australia.

CORRESPONDENCE — Earl H. Harley, MD, Dept of Otolaryngology-Head and Neck Surgery, Georgetown University Medical Center, 3800 Reservoir Rd NW, Washington, DC 20007.

ear is the least common location within the temporal bone. In a report by Denia et al² of 53 temporal bones with osteoma, none was in the middle ear. In that study, the typical locations for extracanalicular osteoma were mastoid, squama, and internal auditory canal. If osteomas occur in the external auditory canal, they must be differentiated from exostoses. Exostoses are felt to be reactive hypertrophy of bone, most notably secondary to cold water exposure. They can be multiple and produce symptoms by obstruction of the ear canal. Osteomas of the ear canal are distinctly different. They are true neoplasms that occur along the tympanosquamous or tympanomastoid suture lines.^{1,2}

While the exact cause of osteomas is not well understood, they are thought to arise from preosseous connective tissue. They consist of mature dense lamellar bone and have haversian canals. There is some evidence that osteomas are of a congenital nature.⁴ Yamasoba et al⁴ suggested this cause because their case presented in conjunction with a congenital cholesteatoma. Further support of the congenital causation was a report by Thomas⁵ of middle ear osteomas

in siblings.

Osteoma occurrence is either syndromic or non-syndromic. Infrequently, osteomas occur as a feature of Gardner's syndrome. Gardner's syndrome is an autosomal dominant disorder that is characterized by multiple intestinal polyps, epidermoid inclusion cysts, fibromas of the skin and mesentery, and osteomas. Osteomas in Gardner's syndrome have a predilection for membranous bones, and as such, the mandible and maxilla are more commonly involved in this syndrome.⁶

Middle ear osteomas may interfere with the ossicular chain mobility and thus produce a conductive hearing loss.⁷ Middle ear osteomas have also been reported to produce vestibular symptoms.⁸

In summary, middle ear osteomas are unusual, benign osseous neoplasms. Middle ear osteomas may be relatively safely observed. In fact, observation is the preferred method of treatment in asymptomatic patients. When there is a functional disorder such as hearing loss or vertigo, judicious removal can be undertaken.^{7,8}

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CLINICOPATHOLOGICAL CONSULTATION

BARRIER BETWEEN THE SUPRAGLOTTIS AND THE GLOTTIS: MYTH OR REALITY?

ALFIO FERLITO, MD

PADUA, ITALY

JAN OLOFSSON, MD, PhD

BERGEN, NORWAY

ALESSANDRA RINALDO, MD

PADUA, ITALY

Controversial opinions on the existence of a "barrier" between the supraglottic and glottic regions of the larynx are reported. Even if the two areas have different embryological derivations, there is no anatomic evidence of a "barrier" that could prevent supraglottic cancer from extending downward to the glottis. Numerous adequate pathologic studies, including whole organ sections, confirm that for advanced cancers, anatomic compartments delimiting the spread of the neoplastic process from the supraglottis to the glottis do not exist. Therefore, supraglottic laryngectomy should be performed not on the basis of embryological considerations, but on the basis of the actual extension of the neoplastic lesion.

It is common knowledge that the parts of the larynx have different embryological derivations.¹ The supraglottis develops from the buccopharyngeal anlage, whereas the glottis and subglottis derive from the tracheobronchial anlage. The supraglottic structures thus emanate from primitive branchial arches III and IV, and the glottic and subglottic structures develop from arches V and VI, but it is difficult to define the exact points of embryological demarcation for the entire larynx.² The different but not very well-demarcated embryological origins of the parts of the larynx have led to the conclusion that this organ is made up of two hemilarynges, which are superposed not only embryologically but also oncologically.³ The supraglottis and the glottis were therefore considered by Bocca et al³ as two different and independent cancerological units. Bocca et al said that "each of these hemilarynges may become invaded by cancer independent of one another, and that the extension of cancer is limited within the boundaries of embryological demarcation."^{3(p1006)} Bocca et al also said, "Even in the most advanced stages of evolution, primary vestibular growths never invaded the floor of the ventricle or the vocal cords...In all cases, the embryological barrier running horizontally through the ventricles firmly withstood the neoplastic invasion..."^{3(p1007)} These assumptions led to erroneous pathologic conclusions on the spread of laryngeal cancer.

In the literature, there are different opinions re-

garding a so-called "barrier" between the supraglottis and the glottis, and horizontal partial laryngectomy is based on the concept that this barrier prevents supraglottic cancer from invading the glottis. Kirchner said, "Operation is based on the concept of a barrier to downward extension of supraglottic cancer, which allows a safe margin of resection through the ventricle and anterior commissure."^{4(p1520)}

The following sentences provide a few examples of the different opinions regarding the existence of a barrier between the supraglottis and the glottis.

Meyer-Breiting and Burkhardt said, "Structures indicating a glottic-supraglottic boundary deriving from the development of the larynx do not exist."^{5(p2)}

Bocca said, "These observations certainly inspired Justo Alonso of Uruguay when he conceived the possibility of guiding the surgeon's knife to follow the barriers nature had erected against tumor invasion."^{6(p261)}

Kirchner stated that "whole-organ sections have failed to demonstrate an anatomical structure within the fundus of the ventricle that could qualify as a barrier to downward spread of cancer from the ventricular band."^{7(p7)}

Million et al said, "Although there is a tendency for supraglottic and glottic lesions to remain confined to their original compartments, there is no anatomic barrier to growth from one area to the next."^{8(p439)}

From the Department of Otolaryngology, University of Padua, Padua, Italy (Ferlito, Rinaldo), and the Department of Otolaryngology-Head and Neck Surgery, University of Bergen, Bergen, Norway (Olofsson). Supported by The Laryngeal Cancer Association, Italy.

CORRESPONDENCE — Alfio Ferlito, MD, Dept of Otolaryngology, University of Padua, Nuovo Policlinico, Via Giustiniani 2, 35128 Padua, Italy.

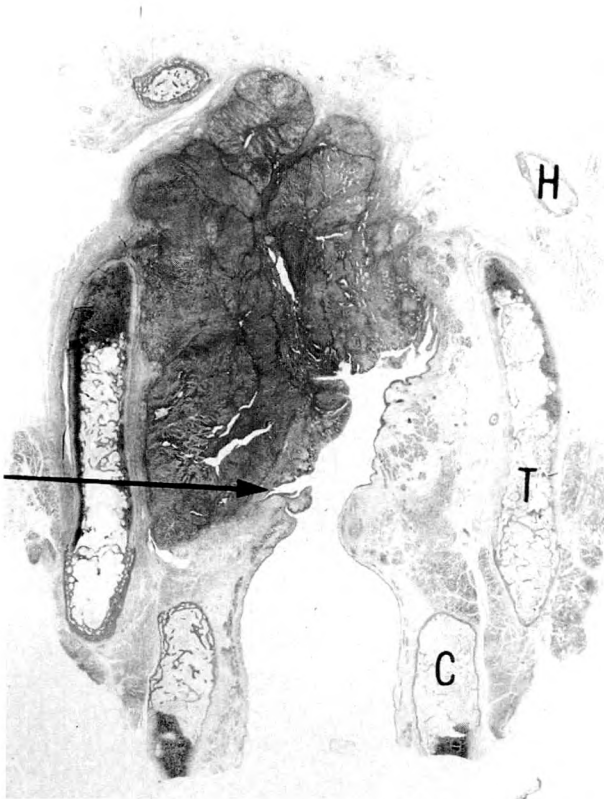


Fig 1. Coronal section at tip of vocal process between middle portion and posterior part of vocal cord in advanced supraglottic carcinoma with pushing margins extending down into vocal cord muscles below inner angle of ventricle (arrow). There is no cartilage destruction. C — cricoid cartilage, H — hyoid bone, T — thyroid cartilage.

Weinstein et al said that “the ventricle functions like a funnel, rather than a barrier, for the spread of cancer in the paraglottic space”^{9(p1133)} and that “if there was a barrier to glottic invasion, one would expect that there would be no correlation between inferior extension of carcinoma and invasion of the glottis, and that perhaps the cancer might extend within millimeters of the glottis without actual invasion. In this series, to the contrary, there was a statistically significant trend ($P = .0001$) for glottic level invasion as carcinoma extended inferiorly along the supraglottic mucosa. Once the cancer reached the glottis, a continuum was noted in which the majority of cases involved the superior surface of the vocal cord and fewer cases were noted as the mucosa was followed inferiorly, with only 10% of cases involving the lateral subglottic mucosa.”^{9(p1132)}

DISCUSSION

Although the concept of a barrier between the supraglottic and glottic regions of the larynx has been frequently repeated in the literature,^{3,10,11} there is no evidence of an anatomic structure that could qualify as a barrier to the downward extension of supraglottic



Fig 2. Coronal section through midportion of vocal cords, showing supraglottic carcinoma involving both sides of supraglottis, but with tumor extension down directly into vocal cord and thyroarytenoid muscle (arrows). Tumor nests can be seen whole way from supraglottic surface down to tumor in thyroarytenoid muscle. C — cricoid cartilage, E — epiglottis, T — thyroid cartilage.

cancer on whole organ sections and in numerous adequate histologic studies.^{9,12-17} Supraglottic cancers may cross the ventricle and extend down the vocal cord (Figs 1-3), sometimes without being visible endoscopically.

The studies of Pressman¹⁸ in the 1950s demonstrated that the larynx is a highly compartmentalized organ, implying the presence of a barrier between the supraglottis and the glottis. But more recently, Welsh et al¹⁹ showed that massive dye infusions in the supraglottis resulted in the spread of dye to the glottic and subglottic regions; therefore, large supraglottic cancers may invade the glottis and subglottis and are not confined to the supraglottic area by some (imaginary) boundary between the supraglottic and glottic regions.

Tumors of the supraglottis expand via different paths in the caudal direction. Hast² pointed out that even if the supraglottis arises from embryological

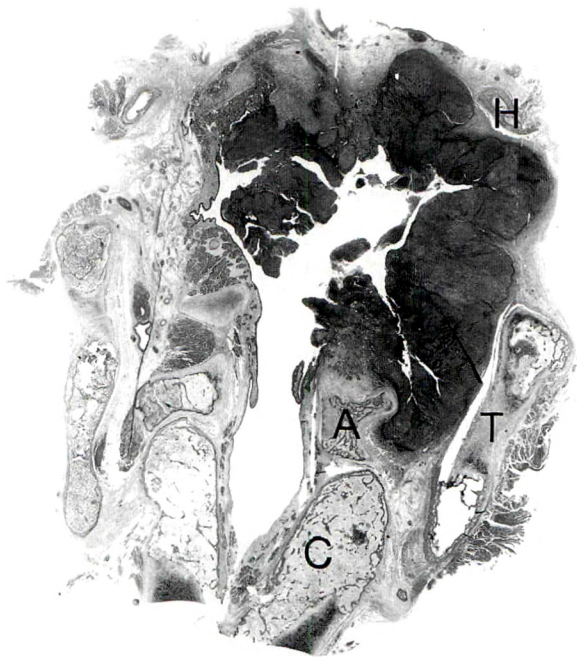


Fig 3. Coronal section through arytenoid region with advanced supraglottic carcinoma occupying both sides of supraglottis, mainly on right side and with extension down laterally to arytenoid cartilage (A). Note also partial destruction of epiglottic cartilage. C — cricoid cartilage, H — hyoid bone, T — thyroid cartilage.

elements different from those of the glottic region, nonetheless it is difficult for the embryologist or the surgeon to define the exact point of "embryological demarcation" between the different regions of the larynx.

We certainly realize that local control achieved by supraglottic laryngectomy in several reports is high where strict criteria for the selection of patients are adhered to. Without doubt, supraglottic laryngectomy is an excellent operation in selected cases of supraglottic carcinomas and can be performed relatively safely. In any case, more advanced supraglottic cancers may extend into the glottic and subglottic regions, both because the laryngeal mucosa represents a continuum, and because the glottis can be invaded via the paraglottic space. To base the validity of supraglottic laryngectomy on embryological considerations would be rather questionable.⁵

If a supraglottic cancer invades the paraglottic space, inferior invasion to the glottis can easily occur.^{5,12,15-17,20,21} This is because the paraglottic space extends into the glottis along the medial surface of

the thyroid cartilage. In the Olofsson and van Nostrand series,¹³ the supraglottic cancers had a fairly high tendency (6 of 25 or 24%) to invade the glottic region.

Han and Yamashita²² investigated the manner of spread of supraglottic cancer by studying whole organ serial sections of surgical specimens, and they found the glottis was involved in 48 cases and the anterior commissure in 12 cases. More recently, Weinstein et al⁹ evaluated the spread of supraglottic cancer to the glottic level by whole organ sections of total laryngectomy specimens from 37 patients with previously untreated supraglottic cancers. Of the 37 specimens, 20 (54%) were noted to have extension of the cancer to the glottic level. The incidence of glottic level invasion from a supraglottic cancer lies somewhere between 20% and 54%.⁹

We think that the reason supraglottic laryngectomy is successful is that the growth of the cancer has not exceeded the supraglottic area, rather than there being any natural obstructive phenomenon. To treat cancer in the best way, we need to know the area involved by the actual tumor and its lymphatic extension.

CONCLUSIONS

1. There is no anatomic structure qualifying as a barrier between the supraglottis and the glottis.
2. The supraglottis and the glottis have different embryological derivations, but exactly where the demarcation line is located has not been established.
3. The oncologic safety of supraglottic laryngectomy is not the result of embryological factors.
4. In particular, more advanced supraglottic cancers have quite a high tendency to invade the glottic region, as observed on whole organ sections.
5. The concept of anatomic compartments delimiting neoplastic infiltration is no longer acceptable for advanced cancers.
6. Supraglottic laryngectomy represents a relatively safe and effective method for treating cancer confined to the supraglottis, regardless of any (unjustified) embryological considerations.

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BOOK REVIEWS

Atlas of Temporal Bone and Lateral Skull Base Surgery

Mario Sanna, Essam Saleh, Alessandra Russo, and Abdel Taibah. Hard cover, illustrated, indexed, 200 pages, 429 illustrations, 1995. Thieme Medical Publishers, New York, NY, \$139.

The authors and publishers have produced a beautifully illustrated and easy to understand atlas of a complicated anatomic site, the temporal bone. The atlas not only serves as an illustrative guide on techniques of otologic and skull base surgery; it also presents enough detail to assist in decision-making.

It is not the medical drawings that distinguish this atlas. The cadaveric anatomic photographs are among the best ever published in otology. These specimens were specially prepared and injected with colored silicone. One can easily identify the anatomic structures. Only at the beginning of the text were the cadaveric illustrations difficult to conceptualize, such as Fig 12.27, and in these illustrations the colored silicone is not utilized. The medical line drawings are neither oversimplified nor lacking in sufficient information. The drawings are accompanied by simple and concise legends that sufficiently convey their intended purpose. More important, the drawings play a supportive role in complementing the cadaveric anatomic specimens.

The main purpose of this atlas is to show the anatomy and lateral skull base approaches to the temporal bone. There are two chapters that are introductory, dealing with general guidelines for drilling and techniques of photographic documentation and a review of mastoid and middle ear surgery. Actually, each approach is preceded by an introductory note on relevant anatomy. Indications, hints, and pitfalls of each approach are simply outlined in a didactic manner.

There is a lack of clarification of the difference between the modified radical mastoidectomy and the modified Bondy technique, if there is any. Better indications for doing a radical mastoidectomy in the presence of sensorineural hearing loss (SNHL) should be conveyed. The authors only mention the indication of SNHL occurring with benign tumors of the middle ear and mastoid. Also, some of the topics in the index appear out of order. The middle fossa approach (MFA) is found in the index as a subtopic of "cranial fossa" and under "craniotomy." Once it is found on pages 88-107, there is excellent coverage of the MFA, with classifications (classic approach to the internal auditory canal, extended MFA, and middle fossa transpetrous approach with removal of the petrous apex), indications, and surgical steps. Familiarity with this book should enhance the learning experience of the neurotologist.

DENNIS G. PAPPAS, MD
Birmingham, Alabama

The External Ear

Frank E. Lucente, William Lawson, and Nelson Lee Novick. Hard cover, illustrated, indexed, 331 pages, 1995. WB Saunders, Philadelphia, Pa, \$93.50.

This book is a successor to Ben Senturia's books *Diseases of the External Ear* (1957) and *Diseases of the External Ear: An Otologic Dermatologic Manual* (1980). In the former, Senturia claimed in the preface that infections of the external auditory canal (EAC) constituted 5% to 20% of outpatient practice, and many more in his (Senturia's) army installation clinics. Present-day numbers of cases of external otitis may not be the same, but diverse diseases of the external canal are such that there is a need for this book. Its depth of coverage is clinically oriented; basic laboratory studies are typically outlined. In the beginning, there are eight pages of color plates; then, scattered throughout the text, the illustrations are black-and-white. I would have preferred the color illustrations be omitted from the front of the book and placed accordingly in the text.

The authors acknowledge that their own experience and techniques may differ from those of others, and not mentioned in the text are conditions that I see. Namely, there is a form of echinosis and blood blister, ie, hemorrhages between the epidermis and subcutaneous layers of skin. Another condition is one of simple erosion — ulcers at the entrance of the bony EAC and without evidence of carcinoma. And there are the conditions of external cholesteatoma, collapse of the meatus from the relaxation of the posterior wall of the cartilaginous meatus falling against the anterior wall, and partial necrosis of the posterior wall producing a spontaneous canal wall down cavity. (This is rare, but worth including for completeness. I see no mention of the acronym TOE, for *Trichophyton*, *Oidiomycetes*, and *Epidermophyton*.)

Especially satisfying are those instructional essays that are distinguished by a blend of medical information and therapeutic techniques. Most of the treatment modalities are well explained, and each chapter is a model of clarity. In 18 chapters on the EAC there is equal distribution of the topics (information) that I would divide into the following: basic science and clinical examination, infectious and allergic conditions, neoplasms, and surgical corrections.

The scheme and contents of this volume are, essentially, in continuity with those of its predecessor compiled in 1980. Yet, the present edition offers some significant departures from its forerunner; although some of the topics are identical, the authors have changed and thus the philosophies of treatment differ from those of the previous edition. This book is well balanced between problems commonly seen, and those not so commonly seen, in the practice of otolaryngology.

DENNIS G. PAPPAS, MD
Birmingham, Alabama

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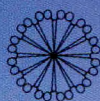
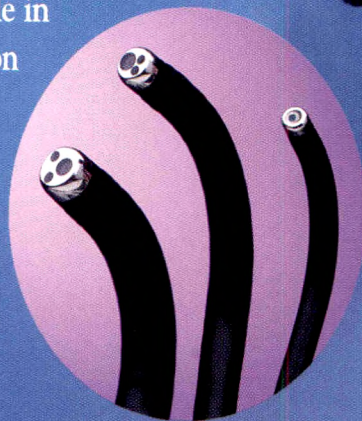
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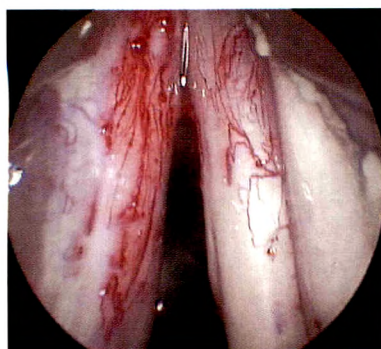
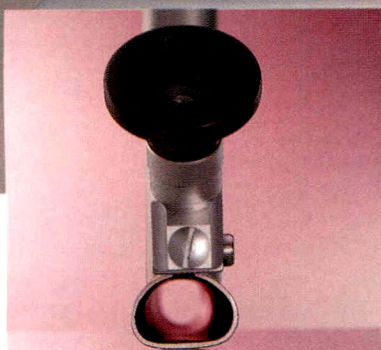
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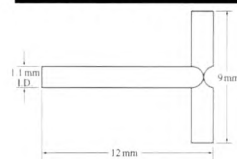
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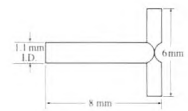
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ELECTROPHYSIOLOGIC CHANGES IN PRETERM NEONATES: AUDITORY BRAIN STEM RESPONSE AND DISTORTION PRODUCT OTOACOUSTIC EMISSIONS

RAFAEL E. QUIÑÓNEZ, PhD

SAN JUAN, PUERTO RICO

MARGIE R. CRAWFORD, PhD

IOWA CITY, IOWA

The purposes of this investigation were to determine 1) if auditory peripheral maturity is present in the newborn; 2) if not, at what age maturational changes occur in the peripheral auditory system from preterm to full-term; and 3) how results of tests used to identify auditory dysfunction in neonates, such as distortion product otoacoustic emissions (DPEs) and auditory brain stem responses (ABRs), change during this period. Longitudinal DPE amplitude and ABR wave I latency measurements were obtained from a single ear of 18 preterm neonates. The DPEs were evoked at f2s of 2, 3, 4, and 5 kHz. The longitudinal data revealed that in general, DPE amplitude increased and ABR wave I latency decreased as a function of postconceptional age. These findings suggest that 1) the peripheral auditory system has not reached maturity in the preterm neonate; 2) maturational changes continue from preterm to full-term; and 3) these changes are reflected in ABR and DPE measurements.

KEY WORDS — auditory brain stem response, otoacoustic emissions, peripheral auditory maturation, preterm infant.

INTRODUCTION

Several investigators have attributed the large degree of variability in neonatal auditory brain stem response (ABR) and, more recently, otoacoustic emission (OAE) measures to maturational changes in the auditory pathway.¹⁻⁹ This variability, along with the inability to define gestational age with less than a ± 2 -week margin of error,¹⁰ makes establishment of normative data for the neonatal population extremely difficult. Furthermore, there exists some disagreement as to whether this variability reflects immaturity of the peripheral portion, central portion, or a combination of both portions of the auditory pathway.^{6-8,11}

Examination of the relationship between peripheral and central auditory maturation has been accomplished by comparing latency and amplitude differences among neonates of different postconceptional ages.^{3,6-9,11} The peripheral pathway is defined as all structures including and peripheral to the cochlea and first-order neurons, and the central pathway, as all structures rostral to the cochlea and first-order neurons. Reduction in ABR wave I latency as a function of postconceptional age has provided evidence to suggest incomplete peripheral maturation at birth.^{3,6,7,11,12} The consensus among several studies is that central immaturity exists not only among preterm neonates,^{3,4,6,8} but also in full-term infants until the age of approximately 18 months.¹³ The issue of peripheral immaturity of the neonatal auditory

system, however, has not been fully addressed.

Samani et al⁷ compared two groups of newborns: preterm infants with a mean postconceptional age of 35 weeks and full-term infants with a mean postconceptional age of 40 weeks. They observed a wide range of wave I latencies among both groups. This range or variability was larger than that observed in adults. They attributed the high variability to the complex and individual processes of maturation. They also found wave I latencies in preterm infants to be more delayed than those observed in full-term infants. They suggested that intersubject differences may be more pronounced in the preterm group than the full-term group, due to maturation of the cochlea and ongoing myelination of the eighth nerve. Zimmerman et al⁹ found wave I latency to be significantly prolonged compared to adult values for up to 4 weeks following full-term birth. They suggest that functional maturity of the peripheral auditory system is complete by this time (4 weeks). However, Schwartz et al⁸ argue against peripheral immaturity on the basis of two findings. First, they were unable to obtain significant differences in wave I latencies and amplitudes among neonates with different postconceptional ages. Second, wave I latency and amplitude measures among these neonates did not differ significantly from those obtained in normal-hearing adults.

The studies mentioned above have one procedural characteristic in common: they all examined postcon-

From the Department of Otolaryngology-Head and Neck Surgery, The University of Iowa Hospitals and Clinics (Quiñónez), and the Department of Speech Pathology and Audiology, The University of Iowa (Crawford), Iowa City, Iowa. This research was supported by the United States Public Health Service, National Institutes of Health (grant RR000359 from the General Clinical Research Center Program).

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CORRESPONDENCE — Rafael E. Quiñónez, PhD, University of Puerto Rico, Medical Sciences Campus, College of Health-Related Professions, Office of Investigations and Project Development, PO Box 365067, San Juan, PR 00936-5067.

ceptional age between subjects. It would have been preferable to examine the same neonates over a specified time period and therefore avoid intersubject variability, as well as inaccuracies in defining postconceptional age.

Some investigators have examined the effects of maturation on OAE amplitude in neonates.¹⁴⁻¹⁷ Chuang et al¹⁵ and Smurzynski¹⁷ were the only investigators to find significant differences in neonates with different postconceptional ages. Chuang et al found that the average transient OAE spectrum measured in 500-Hz bands as a function of postconceptional age increased between 3 and 4.5 kHz. In fact, the most rapid amplitude growth occurred between 37 and 38 weeks of postconceptional age. Smurzynski also observed amplitude increases in both transient emissions and distortion product emissions (DPEs) with increasing postconceptional age, particularly in the 2.8- to 5-kHz region. Chuang et al and Smurzynski differed from others in that they measured the changes in emission amplitude longitudinally. Unfortunately, their data pools were small: 4 and 7 subjects, respectively. The other investigations used larger subject numbers; however, they examined emission amplitude as a function of postconceptional age between subjects, and found no emission amplitude differences.^{14,16}

As mentioned above, one factor that may contribute to the variability and therefore affect the accuracy of screening procedures is maturation.^{3,6,12,18,19} In an attempt to achieve age-specific norms for neonates, the variability associated with maturation needs to be defined. Clearly, central immaturity is present in the neonatal population and continues until the age of 18 months.¹³ However, there is still no consensus as to whether peripheral maturity exists in the neonate. Since the ABR and OAEs are currently being used to identify auditory dysfunction in the neonate, it becomes necessary to understand those changes in both tests that reflect ongoing maturation. This information is important in order to further improve the accuracy and sensitivity of these tests in hearing screening programs.

The present investigation sought to determine the degree of maturation that occurs in the peripheral portion of the neonatal auditory pathway over a limited time period. The peripheral portion of the pathway was defined to include the distal portion of the eighth nerve, the cochlea, and the middle and outer ear. The ABR wave I latency and OAE amplitude were selected to observe the rate and degree of maturation in the periphery.

METHODS

Subjects. Eighteen subjects from the neonatal intermediate care nursery at The University of Iowa Hospitals and Clinics participated in this study. The subjects

selected were at least 33 weeks (± 2 weeks) of postconceptional age at the initial test session. The neonatal intermediate care nursery was used because it provided access to a relatively healthy preterm population. No infants on ventilators participated in this investigation. After the first test session, each infant was given a bilateral otological examination by a staff otolaryngologist to determine middle ear status. If the otological examination revealed no fluid or drainage in the ears, testing was continued. The first test session occurred a minimum of 72 hours after birth. Both ABR and DPE data were collected for 1 ear (chosen randomly) in each subject. Prior to subject participation, parents signed an informed consent statement that was approved by the hospital institutional review board for human experimentation.

Stimuli. The DPE measurements were obtained with an Otodynamics ILO92 PC-based analyzer and probe system. Two simultaneously presented primary tones, f1 and f2, at levels of 65 and 55 dB sound pressure level, respectively, were used to evoke DPEs. The primary tones were presented independently via separate earphones to the ear and were allowed to mix acoustically. Both earphones and a low-noise microphone used to record primary and DPE levels were enclosed in an ILO92 DP infant probe. A rubber tip was placed over the end of the probe assembly system. The DPE recording resolution was 2 points per octave, and the recording time was 240 seconds. The noise floor was determined by calculating the average of 10 adjacent spectral lines for the DPE (2f1 – f2). The frequency f2/f1 ratio used was 1.22. Absolute DPE amplitude was used in the data analysis. The DPE was considered to be present if its amplitude was >3 dB above the level of the corresponding noise.

The ABRs were recorded with disposable silver-silver chloride electrodes affixed to the forehead (active) and ipsilateral mastoid (reference). Both of these electrodes were referenced to a contralateral mastoid ground electrode. The ABRs were elicited by 100-microsecond rarefaction clicks presented at a rate of 23.1/s. These stimuli were delivered via Etymotic ER-3A insert earphones with EAR infant foam plugs at a presentation level of 90 dB normal hearing level. Responses were passed through a 300- to 3,000-Hz band-pass filter, which according to a pilot experiment provided the best wave I morphology. Each average trace consisting of 2,000 samples and a time window of 15 milliseconds was replicated once, and the 2 traces were superimposed for test-retest reliability.

Procedure. Investigators have shown that DPE amplitude measures in preterm neonates¹⁷ and adults²⁰ are highly correlated for both ears of the same individual. Therefore, single-ear testing was done in order to make

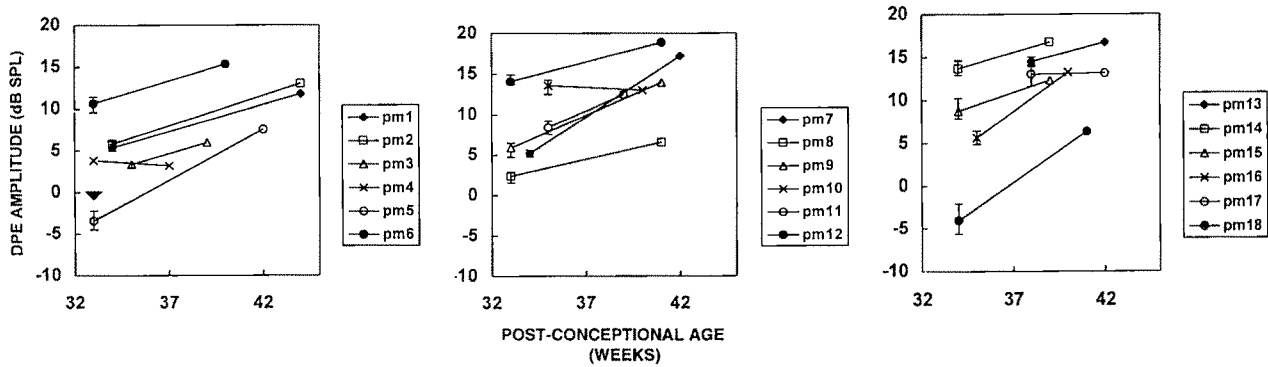


Fig 1. Distortion product otoacoustic emission (DPE) amplitude as function of postconceptional age for f2 of 3 kHz.

the subject pool as independent as possible. The DPE measures were performed on the infants in a quiet room in the intermediate-care neonatal nursery. The ABR measures were performed at bedside in the intermediate-care nursery. The DPEs were recorded at f2 frequencies of 2, 3, 4, and 5 kHz. Each infant was assessed 3 times. A test session consisted of 2 sequential measurements of each response (DPEs and ABRs), starting with DPEs, and lasted approximately 30 to 40 minutes. The first 2 test sessions were performed with no more than 3 days between each session. The 2 sessions were performed close in time in order to determine the degree of within-subject test-retest variability. The final test session occurred approximately 4 to 6 weeks after discharge from the nursery. This session was performed at or close to full term, on the basis of findings that maturation of the peripheral division of the auditory pathway is reached at or shortly after full term.^{3,11,15} Furthermore, it was desirable to allow as much time to elapse as possible between the initial and final test sessions to ensure that the greatest maturational changes, if any, occurred. All testing was performed by the same individual.

RESULTS

The DPEs were reliably recorded above the noise floor at all f2 test frequencies in 17 of 18 (94%) subjects at the initial test session. Subject pm5 exhibited DPEs above the noise floor at f2s of 4 and 5 kHz, but

not at 3 kHz. Furthermore, 10 of 18 (56%) subjects at the initial test session had no DPEs at an f2 of 2 kHz. However, at the final test session, DPEs were present (100%) for all subjects at all f2 test frequencies.

DPE Amplitude and ABR Wave I Latency Data. For all DPE amplitude data, the initial point for all subjects was defined as the mean of 4 measurements obtained during the first and second test sessions. The final point was defined as the mean of 2 measurements taken during the final test session. All error bars on the initial data points (Figs 1-4) represent the range of all 4 measurements obtained during the first 2 sessions.

Figure 1 shows that DPE amplitude increased with postconceptional age for 15 of 18 subjects (83%) at the 3-kHz f2 frequency. A paired-comparison *t*-test between the initial and final group data revealed these amplitude changes to be significant ($t = 5.33$, $p < .05$). Paired-comparison analysis was used to define significant changes between both longitudinal measurements, since each subject served as his or her own control. Figures 2 and 3 show that DPE amplitude increased in 15 of 18 (83%) and 14 of 18 (78%) subjects for f2s of 4 and 5 kHz, respectively. The DPE amplitude changes for both f2s were found to be significant (4 kHz: $t = 4.55$, $p < .05$; 5 kHz: $t = 3.58$, $p < .05$) for the group. Figure 4 shows ABR wave I latency reduction with postconceptional age for all 18 subjects. Paired *t*-test analysis of the group wave I latency data revealed that these reductions were significant ($t = 7.41$, $p < .05$).

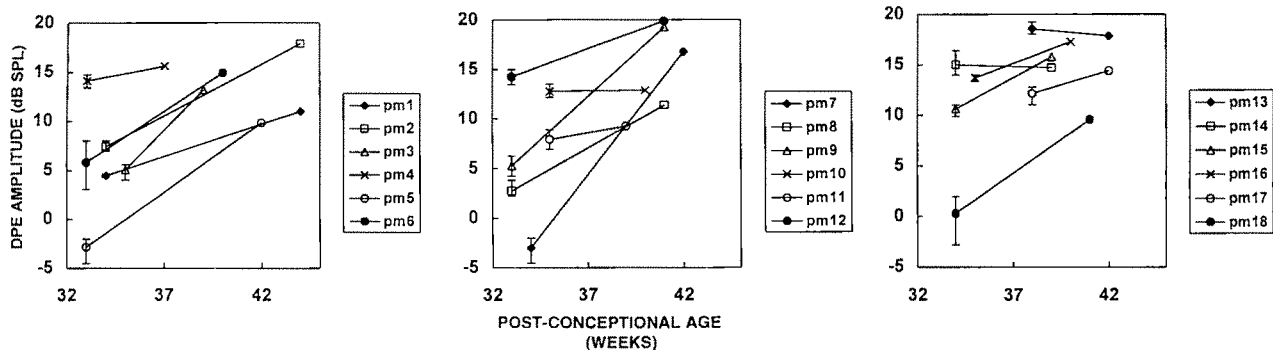


Fig 2. DPE amplitude as function of postconceptional age for f2 of 4 kHz.

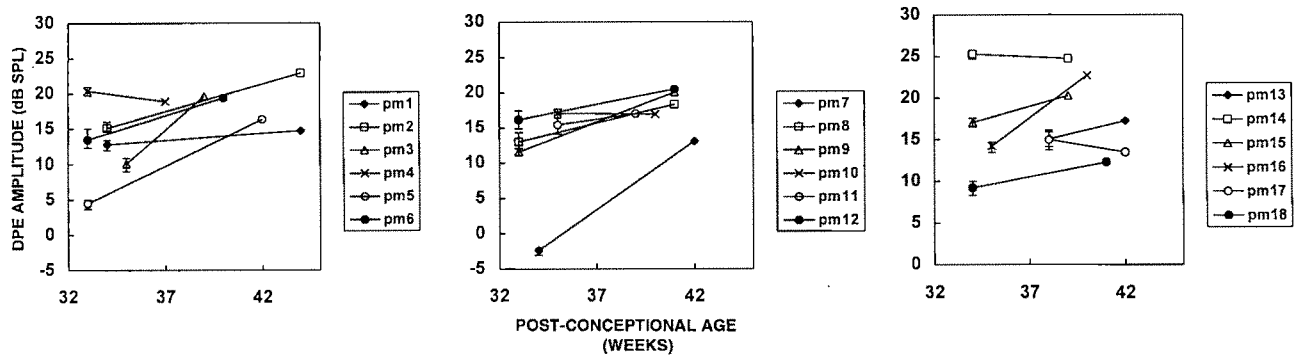


Fig 3. DPE amplitude as function of postconceptional age for f2 of 5 kHz.

Individual DPE Amplitude Changes. Smurzynski¹⁷ defined a significant increase in DPE amplitude with increasing postconceptional age as a difference of >3 dB between the initial and the final test sessions. This criterion was based on the test-retest reliability of DPE amplitude in adults over a 4-week period.²¹ Smurzynski observed that 77% and 85% of all ears tested showed a significant DPE increase (>3 dB) for f2s of 2.8 and 4 kHz, respectively. However, only 46% showed a significant increase at 5.6 kHz. Similar results are observed in the present investigation if the same criterion is applied to the present DPE amplitude data. At 3 kHz, 14 of 18 (78%) subjects showed a significant increase; at 4 kHz, 13 of 18 (72%) showed a significant increase; and at 5 kHz, only 11 of 18 (61%) showed a significant increase. Table 1¹⁷ shows the application of the >3 -dB criterion to the DPE amplitude data of Smurzynski and the present investigation.

Standard errors of measurement (SEMs) were calculated from the 4 measurements obtained during the initial test sessions. This analysis was performed in order to define significant DPE amplitude changes on the basis of the distribution of the present data. The SEM is defined as an estimate of the standard deviation that results from repeated testing of an individual, and so is based on a normal gaussian distribution. Ninety-five percent of all values should fall within ± 2 standard deviations. Therefore, a value greater than ± 2 SEM was chosen as the criterion for significant DPE amplitude

change. The variability of the measurements in the final test session was assumed to be similar to that measured during the initial test sessions, and so no additional analysis was done on these measurements.

Table 2 shows the different criteria that were calculated for all the f2 frequency conditions and the number and percentage of subjects that showed significant DPE amplitude change under these criteria. Significant amplitude change was observed in 83%, 72%, and 78% of all subjects for f2 frequencies of 3, 4, and 5 kHz, respectively. The SEM was not calculated for ABR wave I latency data, since latency was stable and did not vary within subjects across initial measurements.

Changes as Function of Time Interval Between Initial and Final Test Sessions. The present data have shown significant changes in DPE amplitude and wave I latency with increasing postconceptional age. Information regarding the degree and pattern of these changes was examined by investigating the strength of their relationship to the time interval between the final and initial test sessions. The DPE amplitude change was defined as the difference between the final and initial amplitude measurements. The correlation coefficients were statistically significant for each f2 frequency (3 kHz: $r = .68$, $p < .002$; 4 kHz: $r = .69$, $p < .002$; 5 kHz: $r = .51$, $p < .05$). This relationship is such that as the time interval between test sessions increases, so does the change in DPE amplitude. A significant statistical

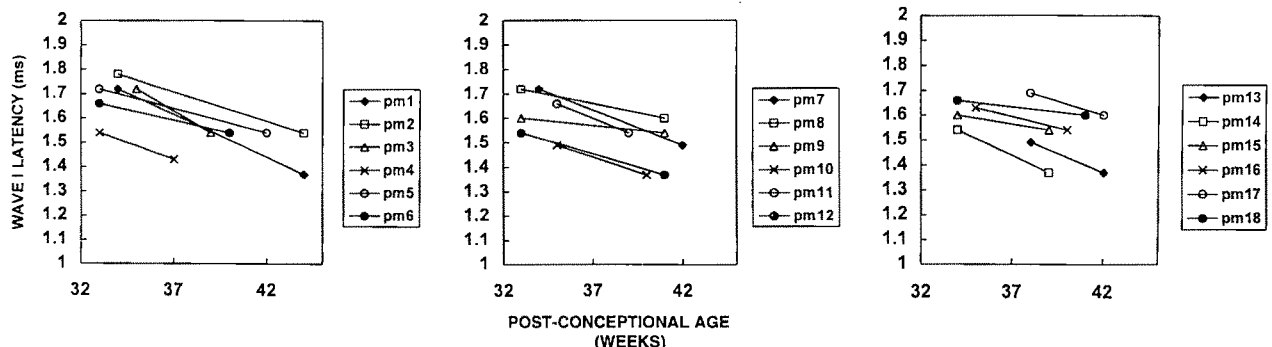


Fig 4. Wave I latency as function of postconceptional age.

TABLE 1. SIGNIFICANT CHANGE IN AMPLITUDE OF DISTORTION PRODUCT OTOACOUSTIC EMISSIONS (>3-DECIBEL CRITERION)

	f2 Frequency (kHz)	Frequency Band (kHz)	Significant Change	
			No.	%
This study (n = 18 subjects)	3		14/18	78
	4		13/18	72
	5		11/18	61
Smurzynski ¹⁷ (n = 13 ears)		2.8	10/13	77
		4.0	11/13	85
		5.6	6/13	46

correlation was also found between wave I latency change and time interval (wave I: $r = .66$, $p < .01$). This relationship is such that as the time interval increased, the wave I latency change also increased.

Correlations Among DPE Amplitudes and Wave I Latency Conditions. The relationship among the three DPE amplitude conditions (f2 of 3, 4, or 5 kHz) was investigated in order to determine whether the relative DPE amplitude change for individual subjects was similar across conditions. In other words, did those subjects who showed large DPE amplitude changes at 3 kHz also show large DPE amplitude changes at 4 and 5 kHz? Regression analysis revealed that these correlations were statistically significant (3 versus 4 kHz: $r = .81$, $p < .002$; 3 versus 5 kHz: $r = .77$, $p < .002$; and 4 versus 5 kHz: $r = .85$, $p < .002$). The relationship between wave I and DPE amplitude changes was also investigated in order to determine whether it was similar for different subjects. This relationship, however, was found not to be significant for any of the f2 conditions.

DISCUSSION

Summary and Explanation of Results. The present investigation measured DPEs above the noise floor at all f2 frequencies in 94% of all preterm neonates at the initial test session and in 100% of all neonates at the final test session. These data are consistent with those of other studies, which found the prevalence of OAEs in preterm infants to be between 93% and 100%.¹⁴⁻¹⁷ Furthermore, DPEs were recorded at all f2 frequencies in 5 of 6 (83%) neonates at 33 weeks of postconceptional age. These findings agree with those of other investigators, who have recorded OAEs in preterm neonates as early as 32 to 33 weeks of postconceptional age,^{14,16,17} and indicate that the active biomechanical properties of the cochlea required for OAE production are already present at this early age.

The present data suggest that the peripheral auditory system in the preterm neonate is not completely mature at birth, but in fact, undergoes maturational-develop-

TABLE 2. SIGNIFICANT CHANGE IN AMPLITUDE OF DISTORTION PRODUCT OTOACOUSTIC EMISSIONS (± 2 -SEM CRITERION)

f2 Frequency (kHz)	± 2 - SEM Criterion	Significant Change (n = 18 Subjects)	
		No.	%
3	1.60	15	83
4	2.00	13	72
5	1.52	14	78

SEM — standard error of measurement.

mental changes through at least full term. Evidence for these maturational changes can be observed in significant increases in DPE amplitude with increasing postconceptional age for 83%, 72%, and 78% of all subjects at f2s of 3, 4, and 5 kHz, respectively. These findings are similar to the significant DPE amplitude growth with increasing postconceptional age reported by Smurzynski¹⁷ in 82% and 72% of all ears tested for high-frequency bands of 2.8 and 4 kHz, respectively. However, the 5-kHz data do not correspond with his 5.6-kHz data, which showed a significant increase in DPE amplitude for only 46% of all ears tested. This difference can be attributed to the criterion used in the present investigation to define significant amplitude change. This criterion was half the size (1.52 dB) of that used by Smurzynski. Further, it is possible that this difference is simply a result of Smurzynski's small data pool (7 subjects) or his small time interval (3 weeks) between initial and final test sessions. Nevertheless, these findings do not agree with those of Bonfils et al¹⁴ and Morlet et al,¹⁶ who have observed no significant amplitude changes as a function of postconceptional age. These two studies performed between-subject comparisons by examining OAE amplitude across various postconceptional ages.^{14,16} This between-subject comparison may mask any possible age-related differences in OAE amplitude if the responses of the target population are highly variable. Due to the lack of OAE investigations on preterm neonates and the small number of subjects examined in these investigations, the degree of preterm OAE variability cannot be ascertained with any certainty. However, data from the present investigation suggest that this variability is large.

Moreover, the inability to define postconceptional age more accurately than within a 2-week margin of error further compounds the inherent variability present in OAE measures of this population. Bonfils et al¹⁴ and Morlet et al¹⁶ collected data across subjects for various postconceptional ages without any longitudinal measurements. Given the large variability of DPE measures across subjects with similar postconceptional ages in the present investigation, it is not surprising that across-subject comparisons showed no significant variations in DPE amplitude.

A significant relationship was observed between DPE amplitude change and the time interval between initial and final test sessions. This relationship showed that larger amplitude changes were observed with larger time intervals. These findings suggest that DPE amplitude changes reflect a maturational-developmental process occurring in the neonatal auditory pathway early in preterm and continuing through late preterm or possibly to full term.

Another interesting observation from examining postconceptional age at the initial test session is that neither of the 2 subjects first tested at 38 weeks of postconceptional age showed a significant increase in DPE amplitude with increasing age. These results are consistent with those of Chuang et al,¹⁵ who observed little to no change in transient-evoked emission amplitude after 38 weeks of postconceptional age. The lack of amplitude change in the older subjects might suggest that the maturational processes have stopped or slowed by this age. These findings, however, are not conclusive, since they are based on only 2 subjects.

The majority of the studies concerning peripheral auditory maturation have examined ABR wave I latency across age and have shown mixed results.¹⁻⁸ These studies, with the exception of one,⁴ have compared wave I latency across subjects, though as cited earlier, the high degree of ABR variability in the preterm population may make it difficult to measure any underlying latency changes.^{7,8} Those investigators that have argued for peripheral immaturity of the auditory system have found a decrease of wave I latency with age until 38 to 44 weeks of postconceptional age, at which point adult values are attained.^{1,2,4,7} These results suggest that maturational-developmental processes in the auditory system are complete once the neonate has reached full term. This finding may partially explain why Schwartz et al⁸ did not observe any differences in wave I latencies between preterm neonates and adults. Their neonatal subjects were late-preterm infants with a mean age of 37 weeks postconception. It is possible that some of those subjects had already reached peripheral maturity prior to the ABR recording.

Wave I results from the present investigation also provided evidence for peripheral auditory immaturity. It was observed that wave I latency decreased with increasing postconceptional age in all 18 subjects. However, it is not clear from wave I latency data when, if at all, maturational-developmental processes are complete. The oldest subjects tested at the initial session were 2 neonates, both 38 weeks of postconceptional age. These 2 subjects showed further reduction in wave I latency when they were tested 4 weeks later in the final test session. The reduction was, however, less than that observed in younger subjects.

The DPE results of the current investigation showed no significant DPE amplitude changes in the 2 oldest subjects. These results might suggest that maturational-developmental changes in the cochlea are complete at approximately 38 weeks of postconceptional age. This finding concurs with those of other investigators that have used wave I latency to determine peripheral maturity.^{1,2,4,7}

The relationship between wave I latency change and the time interval between the initial and final test sessions was examined and was found to be significant, as was the case with DPE amplitude. Wave I latency decreased with an increase in time interval between initial and final test sessions.

The relationship among the three DPE amplitude conditions was examined in order to assess whether individual subjects showed similar amplitude changes across conditions. This relationship was found to be significant. This finding suggests that the underlying developmental process is similar for individuals across the three f2 frequency conditions. The relationship between wave I latency change and DPE amplitude change was also examined, but was not found to be significant. This finding suggests that the underlying developmental processes responsible for the changes in these different measures are not related. However, this finding is not surprising, since changes in wave I latency may reflect not only cochlear development, but also continued eighth nerve myelination.^{3,6,7,11,12}

Interpretation of How Results Reflect Developmental Changes in Distinct Regions of Peripheral Auditory System. The results from the present experiment provide evidence for continued maturational-developmental changes after birth in the peripheral auditory portion of the preterm neonate. The peripheral pathway, however, consists of three distinct regions: the eighth nerve and cochlea, the middle ear, and the outer ear. It is, therefore, unclear to what degree each of these regions contributes to changes in DPE amplitude, DPE latency, and ABR wave I latency across age.

Evidence for Continued Outer and Middle Ear Maturation. Keefe et al²² examined postnatal development of the external and middle ear by measuring ear canal impedance and energy reflectance in infants (1 to 24 months) and adults. They observed that factors such as growth of the ear canal area, as well as length and thickening of the ear canal wall, decrease the impedance characteristics of the external ear. They further observed that in the infant, middle ear compliance was lower and middle ear resistance was higher than in adults. These observations were attributed to the growth of the middle ear cavity. Similar findings were reported by Holte et al,²³ who studied developmental changes in multifrequency tympanograms in infants during the

first 4 months of life. They showed that admittance increased with age and also attributed this to the growth of the middle ear cavity. The combination of both of these developmental changes in the external and middle ear can reduce the power transfer in the middle ear by approximately 8 dB in a 1-month-old infant, compared to that of a 6-month-old infant, for the 3- to 4-kHz range.

Keefe et al²² suggested that these variations in developmental acoustic impedance can greatly influence physiological measurements of auditory development, such as OAE. This suggestion has important implications for the results of the present investigation, since significant changes in DPE amplitude were recorded in the region where Keefe et al²² observed the largest increase of power entering the ear with increasing age. It is reasonable to assume that these DPE amplitude changes may at least partially reflect some of the developmental changes in the outer and middle ear. However, it should be noted that Keefe et al and Holte et al²³ examined infants older than those in the present investigation, and therefore it is not clear whether similar external and middle ear development occurs in preterm neonates.

Evidence for Continued Cochlear Maturation. The outer and middle ear development described by Keefe et al²² in 1- to 24-month old infants may significantly contribute to the DPE amplitude changes in the present investigation. However, it is unclear to what degree these changes occur in the preterm period. Furthermore, the 2 oldest subjects in this investigation did not show significant DPE amplitude changes with age. This finding suggests that there may be some completion of the underlying processes responsible for these amplitude changes. This completion occurs at an age younger than those studied by Keefe et al. Therefore, it is conceivable that DPE amplitude changes may also reflect ongoing cochlear development in the preterm neonate.

Those investigators who have observed prolonged ABR wave I latencies in neonates compared to adults have attributed these results to a lack of functional maturity in the cochlea.^{6,18} Wave I latency in the present investigation decreased with increasing postconceptional age. This finding concurs with that of other investigators, and it also suggests that cochlear development is not yet complete in the preterm neonate.

Furthermore, studies of anatomic changes have shown that cochlear development begins in the midbasal turn and progresses both apically and basally.²⁴ However, behavioral data have shown that responses are first elicited by low- to middle-frequency stimuli.²⁵ For example, Gerber and Dobkin,²⁵ using behavioral observation audiometry in a group of full-term neonates, observed more responses to high-pass-filtered noises with

low cutoff frequencies (0.5 and 1 kHz) than to high-pass-filtered noises with high cutoff frequencies (2 kHz). These findings are inconsistent with the anatomic data and suggest that the apical portion of the cochlea matures first.

Norton et al²⁶ observed findings that support the idea that the basal portion of the cochlea matures first, followed by more apical regions. They studied the auditory development of DPEs in young gerbils and observed an orderly progression in the appearance of DPEs, with responses to high-frequency stimuli occurring first, at 13 to 14 days after birth, and responses to low-frequency stimuli occurring later, at 18 to 19 days after birth. Similar results were observed in the present investigation, in which 10 of the 18 (56%) subjects did not show DPE responses at an f2 test frequency of 2 kHz during the initial test session, but did show a response later, in the final test session.

Evidence for Continued Eighth Nerve Maturation. One of the final stages in the development of the nervous system is the formation of myelin sheaths around the processes and cell bodies of neurons. The onset of myelination in the peripheral processes of the auditory nerve starts to occur around 20 weeks of gestation. However, the formation of myelin is not uniform, and in fact, the rate of myelination and the composition of myelin change significantly during development.

The observed reduction of wave I latency with age in the present investigation and in previous studies^{3,6,7,11,12} provides evidence for continued myelination of the eighth nerve throughout the preterm period. Myelination of small fibers occurs early during the preterm period and is followed by myelination of large fibers toward the end of this period. The progression of fiber myelination can contribute to the latency changes observed in wave I with increasing age. Furthermore, it has been observed that the distal regions of the eighth nerve become myelinated later than the caudal regions.²⁷ It is also known that the generators of wave I are located in the distal portion of the eighth nerve¹³; therefore, it is reasonable to assume that this delayed myelination may be at least partially reflected in the observed reduction in wave I latency with postconceptional age.

CONCLUSIONS

The major findings of the present investigation suggest the following.

1. The DPEs can be measured in preterm neonates as young as 33 weeks (± 2 weeks) of postconceptional age.
2. Peripheral immaturity of the preterm neonatal

auditory system exists, at least through 38 weeks (± 2 weeks) of postconceptional age.

3. The change in DPE amplitude may reflect external and middle ear development during preterm that increases the power transfer to the middle ear with increasing postconceptional age. Stimuli most affected by these changes are probably within the 3- to 4-kHz region.

4. Evidence for continued cochlear maturation is provided by the decrease of wave I latencies with increasing postconceptional age. Furthermore, DPE amplitude changes may not only be strictly related to changes in the middle ear transfer function, but may also be attributable to cochlear development.

5. The changes in wave I latency may also reflect progressive myelination of the eighth nerve during the preterm period.

6. Acknowledgment of the different degrees of pe-

ripheral auditory maturity in the neonatal population is an important first step toward improving the use of ABRs and DPEs in the identification of auditory dysfunction in neonates.

FUTURE STUDIES

Future maturational studies should consider use of longitudinal measurements versus between-subject comparisons because of the high degree of individual variability. This individual variability may prevent the identification of maturational changes when large-group data are examined. Furthermore, peripheral auditory maturation appears to also contribute to the high variability present in physiological testing of auditory function in the neonatal population. This variability can reduce the diagnostic strength of physiological tests, such as OAEs and ABRs, and therefore needs to be addressed, particularly in establishing norms for this population.

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PAROSTEAL OSTEOGENIC SARCOMA OF THE MASTOID BONE

DANIEL R. SEELY, MD, MPH

BELLEVUE, WASHINGTON

GEORGE A. GATES, MD

SEATTLE, WASHINGTON

Parosteal osteogenic sarcoma (POS) is an uncommon surface bone tumor, most often arising from the metaphyseal end of long bones. Involvement of the cranial bones is rare, with only 1 case of mastoid bone POS previously reported in the literature. Two patients with POS of the mastoid are presented, 1 followed up for 25 years after surgical treatment. The presenting signs and symptoms, as well as distinctive radiographic findings, are discussed. Histologic features are also described. Typically, cranial POS appears as a sessile, densely ossified surface growth with radiating bone spicules that blend with surrounding soft tissue. Treatment is en bloc resection, which is curative in most cases.

KEY WORDS — mastoid, osteosarcoma, periosteum, skull neoplasms.

Parosteal osteogenic sarcoma (POS) is a surface bone tumor that comprises 1% of all primary malignant bone tumors and 4% of primary osteogenic sarcomas.¹⁻³ Long bones, particularly the popliteal surface of the femur, are most often affected.¹⁻⁴ Craniofacial involvement is unusual, with the mandible and maxilla being most commonly involved.^{5,6} Calvarial bone involvement has been reported in less than 10 cases, including 1 reported case of mastoid process POS.^{7,8} This report details 2 additional cases of temporal bone POS managed by the same surgeon (G.A.G.); both patients are alive and clinically free of disease after surgical resection. One case has been observed for 25 years after treatment. Presenting signs and symptoms, radiographic features, and histologic findings of POS are discussed.

CASE REPORTS

Patient 1. The patient was 27 years old when she was

first seen in 1971 with a mass behind the right ear that had been present for 20 years. No change had been noted by her husband in the preceding 9 years. The mass had been observed by an experienced oncologist who noted a slight increase in size on serial radiographs from the prior year and referred the patient for biopsy. The patient was in excellent general health. Physical examination revealed a bony hard, immobile mass, 3.5 × 5.0 cm, overlying the mastoid bone and firmly attached to the skull. Radiographs revealed a densely calcified, ossified mass applied to the outer cortex of an otherwise normal mastoid. Ossification and calcification in the superior half of the lesion was sharply defined and contiguous, but in the inferior portion there was some discontinuity of calcification, as well as increased thickness of the overlying soft tissues. A sunburst pattern of radiating bony spicules was evident (Fig 1A).

The lesion was completely excised en bloc on April

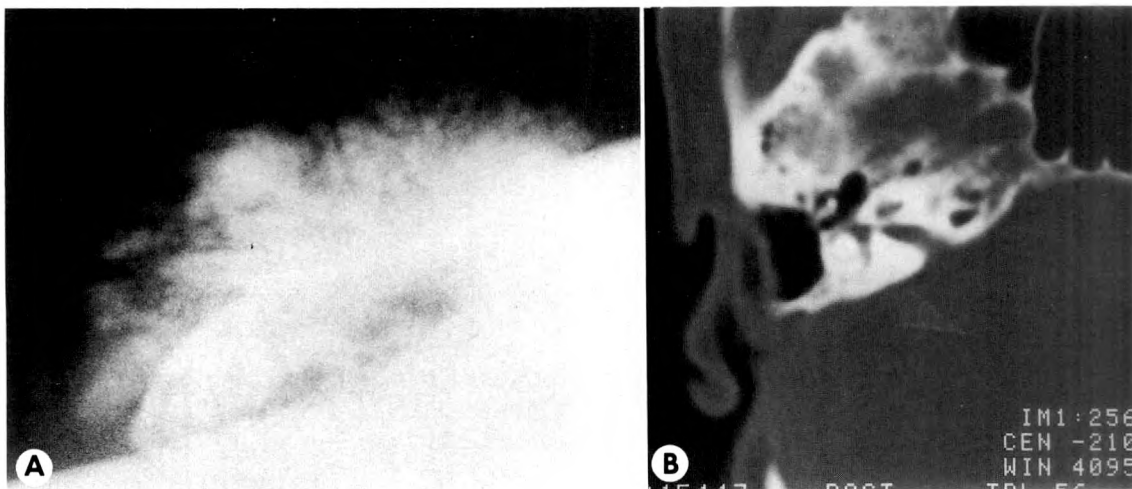


Fig 1. (Case 1) A) Plain radiograph of parosteal osteogenic sarcoma taken at time of diagnosis. B) Computed tomogram taken 20 years after diagnosis.

From the Virginia Merrill Bloedel Hearing Research Center, The University of Washington, Seattle, Washington (Gates).

CORRESPONDENCE — Daniel R. Seely, MD, MPH, Bellevue Ear, Nose, and Throat Clinic, 1201 116th Ave NE, Suite 2, Bellevue, WA 98004.



Fig 2. (Case 2) A) Postauricular mass, upon presentation. B) Computed tomogram of parosteal osteogenic sarcoma. C) Hematoxylin and eosin stain of decalcified specimen showing osteoid within tumor matrix.

1, 1971, through a postauricular incision through which the ear canal, facial nerve, and upper neck structures were visualized. The bony attachments were divided with a cutting bur and chisels, with the normal mastoid air cell system used as a cleavage plane. A diagnosis of POS was made and confirmed by Dr Lauren V. Ackerman of St Louis, Mo. Six months postoperatively there was nodular bone formation at the operative site. A biopsy showed new bone formation that could not be distinguished from recurrent tumor. Reexcision was performed on October 27, 1971, and included the entire mastoid portion of the temporal bone down to dura, the bony ear canal, parotid gland, styloid process, posterior belly of the digastric muscle, and upper portion of the sternocleidomastoid muscle. There was paresis of the facial nerve that recovered completely by 6 weeks. Moderate narrowing of the external auditory meatus required frequent removal of cerumen. Hearing remained normal. Gradual bony growth partially obscured the palpable dural exposure. Four years later a small bony growth of the posterior ear canal was

excised under local anesthesia in order to decrease the problem of ear canal obstruction from cerumen. The patient remained free of clinical disease for the subsequent 20 years. Computed tomography (CT) scans made 20 years after the initial removal showed no evidence of neoplasm without radiotherapy or chemotherapy (Fig 1B). She is alive and clinically well 25 years after diagnosis.

Patient 2. A 39-year-old man presented with a 3-year history of a gradually enlarging right postauricular mass. Over time the mass became painful and was associated with headaches. There was no history of antecedent trauma to the region. Physical examination revealed a 3.0×3.0 -cm mass overlying the right mastoid tip (Fig 2A). Ear examination findings and facial nerve function were normal. A CT scan revealed an exophytic bony lesion with radiating spicules (Fig 2B). There was reactive sclerosis of the outer cortex of the mastoid process. A fine radiolucent cleft was present between the tumor and the mastoid cortex.

The diagnosis of POS was made and an en bloc resection of the tumor was carried out via a postauricular incision. The posterior external auditory canal was skeletonized, the facial nerve was dissected along its vertical portion and at the stylomastoid foramen, and the upper neck structures were visualized. Portions of the posterior belly of the digastric muscle and the sternocleidomastoid muscle were included with the specimen. As in the first patient, the bony attachments were divided with a cutting bur and chisels, with the normal mastoid air cells used as a cleavage plane. Histologic evaluation confirmed the diagnosis of POS and revealed the margins to be free of tumor. A bland stroma was present with normal-appearing fibroblasts (Fig 2C). Osteoid matrix and mature lamellar bone were also present. Flow cytometry revealed diploid DNA and a low proliferative rate, both favorable indicators. Full function of the facial nerve and normal hearing were maintained postoperatively.

DISCUSSION

Parosteal osteogenic sarcoma, or juxtacortical osteosarcoma, is a rare primary neoplasm of bone that arises either in the immediate parosteal connective tissues or in the periosteum itself. It is an indolent tumor that most often occurs in the metaphyseal end of a long bone.¹⁻⁴ More than two thirds of cases arise in the distal femur.

Parosteal osteogenic sarcoma of the long bones typically presents in the third or fourth decade, while most cases of cranial POS present in the second or third decade.^{1-4,7-12} This difference is probably due to the smaller amount of soft tissue overlying the cranial bones, which allows for easier detection of a mass. Our first patient was 27 years old on presentation, but had had a mass since childhood. Both long bone POS and cranial POS have been noted more frequently in women than men.^{1-4,10-12}

Patients usually present with a circumscribed, slow-growing mass that may or may not be tender.⁷ In the series of cranial POSs reported by Kumar et al,⁷ the average duration of symptoms prior to presentation was 9 months, with a range of 1 month to 10 years. Our patients were symptomatic for 20 and 3 years, respectively. The causal role of antecedent trauma is controversial.¹³ Neither of our patients gave a history of prior trauma. It is possible that trauma simply brings attention to a mass that is already present. On examination, POS has a firm, bony consistency, with fixation to the underlying bone and mobile overlying skin. There may or may not be tenderness on palpation.

Parosteal osteogenic sarcoma has characteristic radiologic features that allow for its diagnosis preoperatively with a high degree of certainty. A CT scan

allows the more accurate delineation of these features. Typically, cranial POS appears as a sessile, bosselated, densely ossified surface growth.^{7,14,15} Radiating bony spicules that blend with surrounding soft tissues are characteristic. Reactive sclerosis of the underlying outer table of the skull is usually present, but bone invasion does not occur unless the POS has dedifferentiated. A fine radiolucent cleft may be seen to separate the tumor from the underlying outer table of the skull. Satellite tumor nodules, which have been seen with long bone POS, have not been previously reported in cranial POS.⁷ They have been reported as present prior to surgery, but tend to occur more commonly following surgical resection of the primary tumor.^{11,12} Our first patient manifested recurrent bony nodules that proved to be normal bone on histologic examination. The normal bony regrowth of the mastoid can therefore be a problem in assessing patients for postoperative recurrence.

The histology of cranial POS is indistinguishable from that of long bone POS.^{1-4,11,12} It has a bland appearance that may cause it to be mistaken for a benign lesion by the inexperienced observer. The center of the tumor contains poorly vascularized fibroadipose tissue within compact bone. More peripherally, spindle-shaped fibroblasts with elongated nuclei are evident. Foci of chondroid and osteoid may be found within the tumor matrix (Fig 2C). Parosteal osteogenic sarcoma is known to be capable of dedifferentiation into a high-grade tumor.

The differential diagnosis of cranial POS includes conventional osteosarcoma, chondrosarcoma, sessile osteochondroma, giant osteoma, and organizing scalp hematoma.^{3,11}

The treatment of POS is en bloc resection, which is curative in most cases. Surgical therapy alone was used in both of the cases presented. No adjuvant therapy was necessary. Long bone POS may undergo malignant dedifferentiation in 20% of cases and require more extensive surgery.¹⁶ Dedifferentiation has been reported in 1 case of cranial POS.⁷

Prognosis is much better for POS than for conventional osteosarcoma.^{11,12} Pulmonary metastases from cranial POS have not been reported. In long bone POS, pulmonary metastasis accounts for a mortality of up to 25%.^{2,3,10}

CONCLUSION

En bloc resection of POS of the mastoid cortex appears to be the therapy of choice and may result in long-term disease-free survival without the need for radiotherapy or chemotherapy. Pulmonary and regional metastases were not observed in these cases.

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TRIGEMINAL NEO-NEUROTIZATION OF THE PARALYZED FACE

MACK L. CHENEY, MD

BOSTON, MASSACHUSETTS

MICHAEL J. MCKENNA, MD

BOSTON, MASSACHUSETTS

CAROL WEST, MS

BOSTON, MASSACHUSETTS

CLIFF A. MEGERIAN, MD

WORCESTER, MASSACHUSETTS

MOHAMMED M. ELAHI, MD, MSC

MONTREAL, CANADA

Sporadic reports throughout the literature have documented the spontaneous return of facial function following deliberate intraoperative sacrifice of the facial nerve. Trigeminal reinnervation of the facial muscles has been suggested as one possible mechanism for this occurrence. Evidence for the phenomenon of trigeminal neo-neurotization has been documented experimentally. The case of a 62-year-old woman who underwent total left parotidectomy with transection of a large facial nerve segment is presented in order to provide further clinical evidence supporting trigeminal neo-neurotization of the facial nerve. Despite the lack of any efforts to reinnervate the patient or graft the facial nerve defect, the patient spontaneously developed return of facial function. Postoperative clinical and electrical testing in this case supports trigeminal-facial reinnervation as the cause for return of facial function. The case report is summarized with a brief discussion, and the relevant literature is thoroughly reviewed.

KEY WORDS — facial nerve, facial paralysis, neo-neurotization, parotidectomy, trigeminal nerve.

INTRODUCTION

Scattered reports have documented spontaneous return of various degrees of facial movement following the deliberate intraoperative sacrifice of the facial nerve in cases without any attempt at neuro-rhaphy.¹⁻¹² This clinical observation has led to much speculation regarding the causative mechanism for such recovery of facial nerve function. A number of differing hypotheses on the cause of this phenomenon have been both advocated and refuted by as many authors, resulting in a lack of consensus within the surgical community. As a result of this controversy, four major theories have come to the forefront as potential explanations for this phenomenon: "aberrant unknown pathways,"^{11(p643)} open-field regulation, ipsilateral trigeminal innervation, and contralateral facial innervation.¹²

The following case report demonstrates both clinical and electroneurographic evidence supporting ipsilateral trigeminal neurotization of the facial musculature following facial nerve sacrifice in the absence of a formal facial nerve reanimation procedure. The case history and electroneurographic analysis are presented, followed by a review of the relevant literature.

CASE REPORT

A 62-year-old woman had previously undergone a

left superficial parotidectomy for the management of a low-grade mucoepidermoid carcinoma in 1959. At that time, the integrity and function of the facial nerve were preserved. The patient remained asymptomatic until January 1991, when she presented to an outside institution with the gradual onset of left-sided otalgia and facial paralysis. The physical examination revealed weakness in all divisions of the facial nerve. The remainder of the physical examination findings, other than the presence of an ipsilateral parotid mass, were unremarkable. A computed tomography (CT) scan delineated a hyperdense mass of the left parotid gland, measuring approximately 3.8 cm, with no evidence of metastatic disease. A CT-guided biopsy yielded cytologic evidence of a low-grade mucoepidermoid carcinoma.

Complete surgical resection necessitated a total parotidectomy and sacrifice of the facial nerve due to tumor involvement of the nerve, both within and proximal to the parotid region. The main trunk of the seventh nerve was therefore identified, isolated, and transected at the level of the stylomastoid foramen. The tumor was completely resected along with a 7.5-cm segment of the facial nerve, and negative surgical margins were achieved. Partial resection of the body of the ipsilateral masseter muscle was necessary for complete tumor removal. No attempts were made to reanastomose or graft the facial nerve, nor were facial nerve suspension techniques employed. Postopera-

From the Department of Otolaryngology and Laryngology, Harvard Medical School (Cheney, McKenna, Megerian, West), and the Departments of Otolaryngology (all authors) and Audiology (West), Massachusetts Eye and Ear Infirmary, Boston, Massachusetts.

CORRESPONDENCE — Mack L. Cheney, MD, Dept of Otolaryngology, Massachusetts Eye and Ear Infirmary, 243 Charles St, Boston, MA 02114.

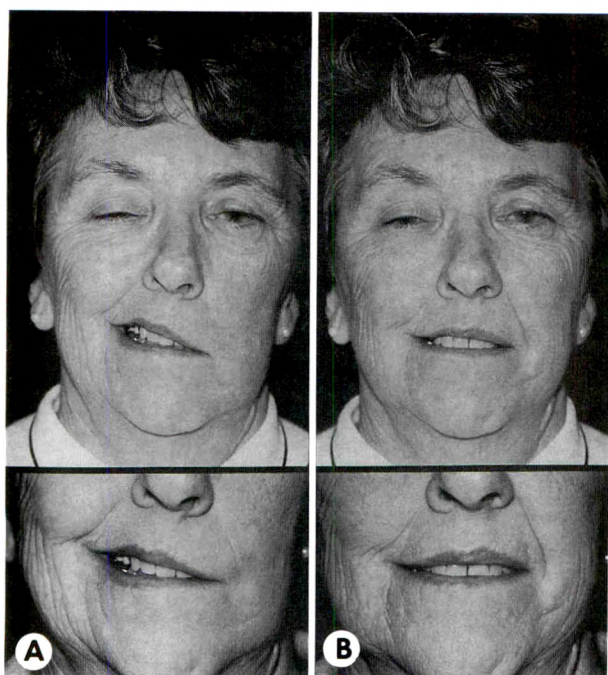


Fig 1. Facial paralysis 8 months after total parotidectomy. A) Facial movement generated by spontaneous smile (top), and close-up of spontaneous smile (bottom). B) Facial movement when "biting down" on back teeth (top), and close-up of smile when "biting down" (bottom).

tively, there was complete paralysis of the left side of the face. A 1.2-g weight was inserted into the left upper eyelid to minimize ocular complications. Facial nerve reanimation surgery was deferred because of the patient's reluctance to undergo further surgery.

Approximately 8 months postoperatively, the patient noted spontaneous left-sided oral commissure movement when chewing. She was subsequently seen in the Facial Nerve Center at the Massachusetts Eye and Ear Infirmary for evaluation and management of her facial paralysis. Careful neurologic examination at that time demonstrated organized left facial movement when the patient was asked to bite on the posterior molars (Fig 1).

METHODS

Electroneurography and blink reflex testing were performed and surface electromyography (EMG) recordings were made with a modified Amplaid MK7 Evoked Potential System. Surface recording electrodes (6-mm diameter, 18-mm spacing) were placed under each eye and along each nasolabial groove. The eyelid electrodes were placed with the active electrode directly under the pupil. The reference electrode rested on the zygomatic region, coplanar to the active electrode. Recordings from the perioral muscles were made from electrodes placed in the nasolabial groove, with the active electrode positioned immediately lateral to the nasal ala. Appropriate ground

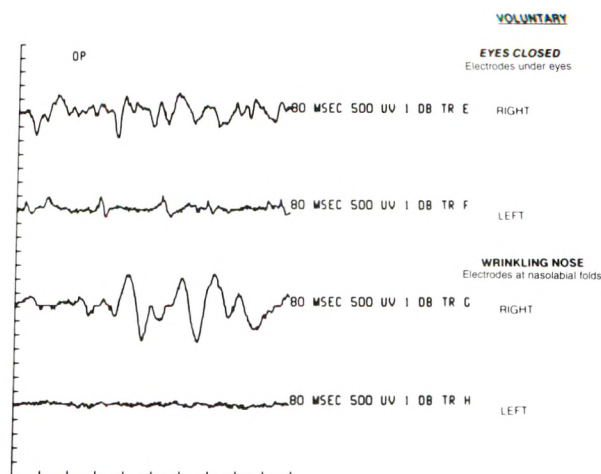


Fig 2. Electromyographic activity is absent from left nasolabial fold when patient attempts to smile or wrinkle her nose.

electrodes were utilized. For electroneurography, a 200-microsecond, 30-mA pulse was delivered through a bipolar electrode with the anode positioned at the stylomastoid foramen and the cathode positioned anterior to the earlobe. The blink reflex was recorded from the electrodes positioned anterior to the earlobe. Electromyographic recordings were made for forced eye closure, nose wrinkling, tooth clenching, and smiling.

All measures were performed before and 20 to 30 minutes after 3 mL of 2% Xylocaine was injected into the region of the left stylomastoid foramen to block any response that may have come from the regenerated fibers of the ipsilateral facial nerve. A similar amount of Xylocaine was then injected into the right stylomastoid foramen to block responses of the left orbicularis oris muscle resulting from cross-innervation by the right intact facial nerve. Informed consent was obtained for electrical studies as well as all necessary nerve blocks.

RESULTS

Preinjection. The blink reflex was present for each side. For the affected, left, side the latency of the early component was delayed (15.52 milliseconds for the left side compared with 12.96 milliseconds for the right side) and the phase reversed relative to the normal response from the right side. The amplitude of the response from the left side was reduced when compared to the right. Electromyographic activity was present from the left orbicularis oculi muscle upon voluntary eye closure, and from the left but not the right orbicularis oris muscle when the patient clenched her teeth. Electromyographic activity was absent from the nasolabial fold when the patient attempted to smile or wrinkle her nose (Fig 2).

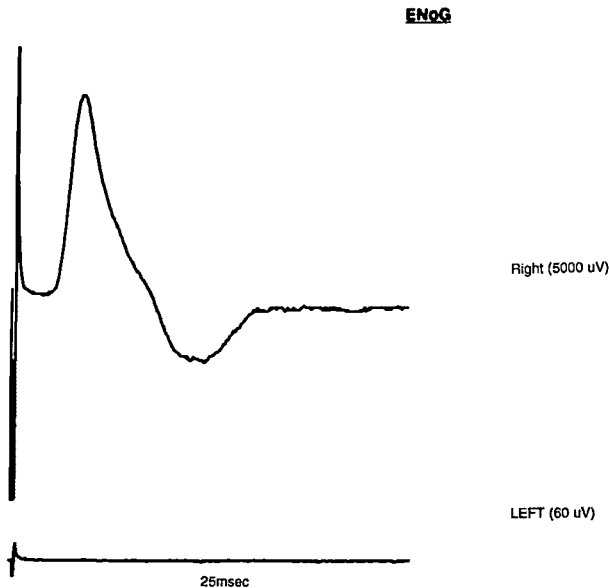


Fig 3. Electroneurography revealed no response from left perioral muscles in response to electrical stimulation at stylomastoid foramen. Compound action potential from right side was normal, with 5.2- μ V peak-to-trough amplitude.

Electroneurography revealed no response from the left perioral muscles in response to electrical stimulation at the stylomastoid foramen. The compound action potential recorded from the right side was normal, with a 5.2- μ V peak-to-trough amplitude (Fig 3), and no contralateral facial movement was elicited upon stimulation of the right stylomastoid foramen.

Postinjection. Following injection of Xylocaine into the left stylomastoid foramen, the blink reflex for the left side was reduced in amplitude when compared to the preinjection condition. Electromyographic activity was absent from the left eye upon attempts at bilateral eye closure. Electromyographic activity of the left orbicularis oris muscle was present and unchanged from the preinjection condition during tooth clenching (Fig 4). The decrease in blink reflex amplitude and disappearance of EMG activity for eye closure on the left side following Xylocaine injection suggest either persistence or regeneration of upper-division facial nerve fibers. The persistence of left orbicularis oris function and EMG activity for tooth clenching on the left side following the administration of Xylocaine to the ipsilateral stylomastoid foramen indicates the absence of contribution from the ipsilateral facial nerve for that function. The lack of left-sided perioral function or EMG activity following electrical stimulation of the contralateral foramen indicates that cross-facial reinnervation is not contributory to recovery of function. In addition, the persistence of left-sided function following blockade of the contralateral stylomastoid foramen further indicates the absence of motor contribution from the

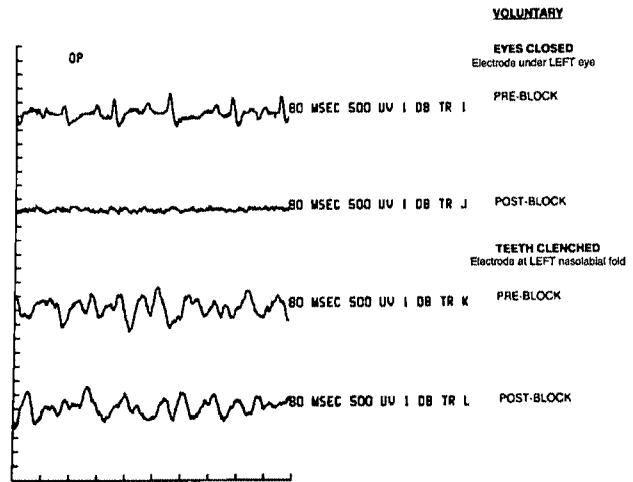


Fig 4. After injection of Xylocaine into left stylomastoid foramen, electromyographic activity was absent from left eye musculature (eyes closed, post-block). However, activity of left orbicularis oris muscle was unchanged in postinjection period during tooth-clenching (teeth clenched).

right facial nerve. These data support the hypothesis that innervation of the muscles of lower facial expression has been assumed by the ipsilateral trigeminal nerve.

DISCUSSION

In the highlighted case, extensive manipulation and partial resection of the masseter muscle during total parotidectomy likely contributed to conditions favorable to trigeminal neo-neurotization of the ipsilateral perioral musculature. In 1955, Conley¹⁰ observed the recovery of facial movement following excision of the facial nerve despite the fact that no effort had been made to perform a nerve graft. In the report, he described two cases of extensive sarcomatous tumors that required resection of 5-cm facial nerve segments. He reported that within 12 months there was spontaneous recovery of facial function to within 90% of normal.¹⁰ The mechanism responsible for this observation was not immediately apparent. In 1963, Conley et al¹¹ documented similar findings in a group of five patients exhibiting the same phenomenon. They concluded from their analyses that open-field regeneration from the proximal facial nerve segment was most likely responsible for the functional return of the paretic muscles. The supposition that reinnervation of the facial musculature was a result of spontaneous regeneration from the proximal facial nerve segment was supplemented with the concept of collateral reinnervation from intact peripheral nerve segments, and was supported by numerous other studies.^{9,12-16} In the present study, however, the possibility of contralateral reinnervation was ruled out after blockade of the contralateral facial nerve with Xylocaine failed to abolish ipsilateral orbicularis

oris function. As suggested by Fisch et al,¹⁷ this test is important when investigating a case of neo-neurotization.

Conley et al¹¹ had based their hypothesis on the fact that local anesthetic injections to the maxillary and mandibular divisions of the trigeminal nerve did not interfere with the reinnervation of the facial musculature in three of five patients tested. Also in support of proximal facial neurotization was knowledge that two patients in this series had undergone concomitant extirpation of the ipsilateral mandibular nerve, which led Conley et al to theorize the plausibility of an aberrant neuronal route separate from the main divisions of the fifth and seventh cranial nerves, presumably under the control of the seventh nerve nucleus. They also speculated that the greater superficial petrosal nerve, by way of its connections with the sphenopalatine ganglion and maxillary nerve, may contribute to this phenomenon. Conley et al,^{11,18} however, also introduced evidence that supported the involvement of the trigeminal nerve in the spontaneous reinnervation of the mimetic muscles, based on observations noted in two patients in whom paralysis of facial movement followed anesthesia of the maxillary and mandibular nerves.

The potential for trigeminal neo-neurotization of paralyzed facial muscles was first hypothesized by Lexer and Rosenthal in the early 1900s.¹⁹ Martin and Helsper¹ are generally credited with the first documented case series supporting the role of the trigeminal system in spontaneously reinnervating the facial musculature. This series noted spontaneous return of facial function in 28.5% of patients who had undergone extratemporal seventh nerve transection of segments ranging from 2.5 to 5.0 cm in length, without interposition nerve grafting or repair.¹

Regrowth of seventh nerve motor fibers was felt to be a less-than-adequate explanation for this occurrence, due to the unlikelihood of reestablishing nervous connections across such unbridged defects.¹ Martin and Helsper further stated that if reinnervation had been achieved via the severed facial nerve, "then a re-excision of the parotid area for recurrence would obviously interrupt such neural pathways, and whatever active motion had been re-established would immediately be lost."^{1(p722)} This comment was supported when two patients had a second excision for recurrent tumor that temporarily interfered with the original gains in facial movement, which returned to original levels in a matter of weeks.

Martin and Helsper reasoned that the establishment of new motor pathways linking terminal trigeminal branches with their facial nerve counter-

parts, primarily through the motor fibers of the mandibular nerve via the masseter muscle, effectively explained the phenomenon of spontaneous trigeminal reinnervation.^{1,20} Additionally, they felt that re-education of voluntary motor impulses from the cortex to the paretic muscles, via the trigeminal nerve, may be the physiological response to seventh nerve transection.¹ This theory was supported experimentally by Erlacher,²¹ who demonstrated that a paralyzed muscle would regenerate by neurotization.

This elaborate theory generated great controversy and debate in the medical literature of the time.^{9,13-16,22,23} Kettel¹⁵ of Denmark was a particular opponent to the theory put forth by Martin and Helsper,^{1,20} calling into question the validity of the report. Kettel was of the opinion that the theory of trigeminal reinnervation was a "flagrant contradiction to all physiological and neurological laws hitherto accepted."^{15(p1,069)} Huber and Highson²⁴ discounted any contribution from the motor branch of the trigeminal nerve to the facial musculature based on physiological experimentation, in support of Kettel's claims. Kettel believed that facial nerve regeneration or the decussation of fibers across the midline was a more feasible explanation for what Martin and Helsper had observed. Buchthal²³ argued that myoneurotization from injury to the motor component of the trigeminal nerve during parotid surgery was responsible. Pollock et al¹³ successfully proved, based on histologic examinations, that regeneration of seventh nerve fibers was commonplace following peripheral motor nerve injuries, in contradiction to Martin and Helsper. Furthermore, in 1966, Boyle failed to demonstrate collateral innervation of the facial muscles by the fifth nerve in his thesis investigating facial nerve regeneration in monkeys. He concluded that "spontaneous recovery of function, when it occurs, is due to random regeneration of axonal elements of the facial nerve and not to vicarious function from the trigeminal nerve."^{9(p1,940)} Boyle's experiments had been preceded by the work of James et al¹⁴ 6 years earlier, who observed similar parameters through facial nerve resection in puppies, in order to corroborate the claims of Martin and Helsper.^{1,20} Their investigation also concluded that the facial nerve was responsible for facial reinnervation as opposed to the trigeminal nerve.¹⁴ Under-scoring this result, they professed the tremendous regenerative potential of the facial nerve as the significant factor in spontaneous reinnervation.

In Martin and Helsper's supplementary report²⁰ to their original article,¹ further evidence in support of their theory is provided. The case report of a patient who developed spontaneous recovery of facial function following segmental facial nerve excision, who

had also undergone reexcision for recurrent tumor, was reiterated. The recovery initially achieved was not interrupted by this second procedure, but more important, the patient subsequently developed neuralgia of the mandibular division of the fifth nerve. The patient agreed and submitted to "alcohol neurolysis"^{20(p541)} in order to alleviate the facial pain. Following the procedure, the patient not only suffered facial anesthesia in the mandibular nerve distribution, but also exhibited an immediate loss of all recovered function in the facial musculature. Recovery to the same level of function as prior to the "alcohol neurolysis" was eventually achieved 9 months later, and this was accompanied by the return of sensation to the previously anesthetized mandibular region. With this added case report, Martin and Helsper²⁰ conclusively contended that "spontaneous recovery of motion in the facial musculature in cases of segmental operative loss of the seventh cranial nerve, is due to the establishment of new motor pathways by way of the fifth cranial nerve."^{20(p541)}

As alluded to above, several publications^{2-7,20,25-27} have appeared throughout the medical literature in support of the theory initially described by Martin and Helsper.¹ Silverstein et al⁷ described a patient with a facial teratoma that resulted in obliteration of the vertical segment of the facial nerve, but in whom spontaneous return of facial motion eventually resulted. An anesthetic block of the mandibular nerve produced complete facial paralysis on the affected side. In 1972, Trojaborg and Siemssen³ concluded from the findings in four patients with spontaneous facial muscle recovery that reinnervation was due to the misdirection of regenerating axons into nearby facial muscles from a masticator nerve injured at the time of operation. This concept of "myoneurotization" led Trojaborg and Siemssen to speculate that partial removal of the masseter muscle, with the resultant trauma to terminal nerve branches, enhances spontaneous return of facial motion via the trigeminal system.

Norris and Proud described unexpected facial innervation in four patients who had practiced tooth-clenching and grimacing, implying the possibility that perhaps "re-education of dormant trigeminal nerve fibers might have facilitated the rehabilitation that was achieved."^{6(p211)} More recently, DeLacure et al presented a case in which they asserted "unequivocal clinical evidence of trigeminal-facial cross-innervation,"^{4(p1,081)} acknowledging, however, that a nerve graft had been performed and was responsible for part of the facial innervation. Of interest, they speculated that competition for denervated facial muscles had occurred, with domination by the more proximal trigeminal nerve.

The potential pathways for spontaneous reinnervation of the facial musculature by the trigeminal nerve were succinctly summarized by Cerny and Steidl.² The authors attributed a case of unexpected facial reinnervation to dual innervation of the buccinator muscle. They refuted the possibility of seventh nerve regeneration, claiming that "it is unlikely that outgrowth sprouts of the main [facial] nerve trunk could persist...and penetrate up to the paralyzed facial muscle."^{2(p103)} Cerny and Steidl pointed to the variable course of the masticator nerve, and the assumptions made by Silverstein et al⁷ and Mundnich²⁶ regarding their estimate of a nerve V-VII anastomosis present in upward of 15% of individuals, in support of their claims. Electromyographic studies were performed on the patient; nevertheless, the anatomic pathways utilized were not clear.

In 1967, Gasser²⁸ published his much-celebrated work on facial nerve development, in which he successfully detailed nerve morphogenesis, devoting specific attention to the peripheral nerve network. Of particular significance was Gasser's description of the existence of "numerous connections...between the peripheral branches of the facial nerve and the branches of all three major divisions of the trigeminal nerve."^{28(p53)} This concept was in accord with Bowden and Mahran,²⁵ who further alleged that these communications consisted of trigeminal fibers that accompanied the facial nerve branches.

A truly comprehensive review on the issue of trigeminal-facial nerve communications was assembled by Baume⁵. He attributed teleological significance to an "overlapping, dual motor nerve supply by the motor trigeminal, as well as facial"^{5(p41)} nerve systems, in conferring a selective advantage to humans. Baume maintained that the terminal communications between the trigeminal and facial nerves are present to facilitate motor reinnervation in case of facial nerve transection. The author also cited embryological evidence of "misrouted facial fibers coursing with the motor route of the trigeminal...subsequently rejoining the facial nerve branches via the various V-VII communicating rami."^{5(p42)}

Bowden et al^{25,27} and Baume⁵ also point out that the principal trigeminal-facial communications occur via the mandibular division of the fifth nerve. Furthermore, Baume⁵ noted that the clinical observation of return of facial muscle function, initially manifesting at the angle of the mouth, furnishes evidence in this respect.

CONCLUSION

The clinical significance of this case is related not only to a limited number of case reports, which we

have attempted to review, but also to the fact that if this phenomenon is predictable, it may be very useful in the management of patients with facial paralysis.²⁹ A cornerstone to early rehabilitation of the paralyzed face has been the use of temporalis muscle transposition.^{30,31} It has been our experience that in selected cases, the long-term movement generated by this technique is more organized and substantial than might be predicted by either facial nerve regeneration

or segmental temporalis muscle transfer. It is possible that trigeminal myoneurotization of the facial musculature is responsible for these clinical findings following dynamic facial reanimation.³¹ If this could be further validated, it may lead to the development of new techniques to promote trigeminal neo-neurotization in the early stages of facial muscle paralysis when there is no potential for facial nerve regeneration.

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INVISIBLE CULPRIT: INTRALABYRINTHINE SCHWANNOMAS THAT DO NOT APPEAR ON ENHANCED MAGNETIC RESONANCE IMAGING

ROSS I. S. ZBAR, MD

IOWA CITY, IOWA

ASHRAF KHAN, MD, MRCPATH

WORCESTER, MASSACHUSETTS

CLIFF A. MEGERIAN, MD

WORCESTER, MASSACHUSETTS

JAY T. RUBINSTEIN, MD, PhD

IOWA CITY, IOWA

Intralabyrinthine schwannoma (ILS) is an infrequent tumor that arises in isolation within the periphery of the temporal bone. Only 32 cases have been reported to date in the literature, of which 12 were discovered at autopsy. Prior to the advent of gadolinium-enhanced magnetic resonance imaging (Gd-MRI), only 1 ILS had been diagnosed preoperatively. However, after Gd-MRI became a common modality, 5 ILSs were imaged. Two additional cases are reported that were discovered during labyrinth-destructive surgery despite normal Gd-MRI findings. Possible explanations for and potential ramifications of nonenhancing ILS are discussed.

KEY WORDS — gadolinium, intralabyrinthine schwannoma, magnetic resonance imaging.

INTRODUCTION

Vestibular schwannoma (acoustic neuroma) is a slowly growing, solitary, benign tumor arising from the perineural Schwann sheath cells of the vestibular portion of the eighth cranial nerve. These tumors can arise anywhere along the course of the vestibular nerve peripheral to the Schwann-glia junction. The growths are composed of either compact sheets of Schwann cells (Antoni type A) or distinct single-cell fascicles (Antoni type B). The majority of these tumors arise from the intracanalicular portion of the superior vestibular nerve, but they also commonly arise from the inferior vestibular nerve. Less frequently, acoustic neuromas may occur in isolation within the temporal bone, eg, the cochlea, semicircular canals, vestibule, or some combination of these structures. These isolated peripheral acoustic neuromas are known as intralabyrinthine schwannomas (ILSs). These ILSs should not be confused with acoustic neuromas that demonstrate lateral extension to the inner ear structures. True ILSs are found in patients with von Recklinghausen's disease.¹ To date, fewer than 20 reported cases of isolated ILSs in living patients without neurofibromatosis have been recorded.²⁻⁸ There have been an additional 12 cases recorded at autopsy.²

The symptoms of ILS include unilateral sensorineural hearing loss, vertigo, and tinnitus. Vertiginous episodes associated with ILS are severe enough to actually mimic Meniere's disease, and they differ from the vague unsteadiness caused by intracanalicular schwan-

nomas. This is likely because ILSs arise from distal portions of the vestibular nerve and therefore do not cause degeneration of the vestibular (Scarpa's) ganglion. Thus, abnormal nerve discharges due to tumor are transmitted to an intact central vestibular nerve, thereby mimicking Meniere's disease.⁹ This accounts for the frequent misdiagnosis of Meniere's disease in patients with ILS. Intracranial imaging is paramount in obtaining the proper diagnosis.

The first schwannoma discovered at surgery that arose from the periphery of the cochleovestibular system is credited to Wanamaker¹⁰ (1972). This tumor was not visualized preoperatively, despite tomograms of the internal auditory canals (IACs) and a pneumoencephalogram of the posterior fossa and ventricles. Wanamaker discovered this ILS during transcanal destructive labyrinthectomy in a patient presumed to have Meniere's disease. Unfortunately, Wanamaker did not comment about retrospective review of the films, nor about how well the inner ears were visualized. Later that same year, the first case of radiologic localization of an intracochlear schwannoma was reported. Karlan et al¹¹ obtained a tomogram that demonstrated "nonvisualization of the basilar turn of the cochlea" that during subsequent surgery was found to be a schwannoma of the cochlea. This case was the only radiographically localized peripheral schwannoma for 20 years, until magnetic resonance imaging (MRI) became commonly available. The difficulty in preoperatively imaging ILS prompted Babin and Harker to write in 1980 that "small intralabyrinthine neurinomas remind us that a normal

From the Department of Otolaryngology-Head and Neck Surgery, The University of Iowa Hospitals and Clinics, Iowa City, Iowa (Zbar, Rubinstein), and the Departments of Otolaryngology-Head and Neck Surgery (Megerian) and Pathology (Khan), University of Massachusetts Medical Center, Worcester, Massachusetts.

CORRESPONDENCE — Jay T. Rubinstein, MD, PhD, Dept of Otolaryngology-Head and Neck Surgery, The University of Iowa Hospitals and Clinics, 200 Hawkins Dr, Iowa City, IA 52242.

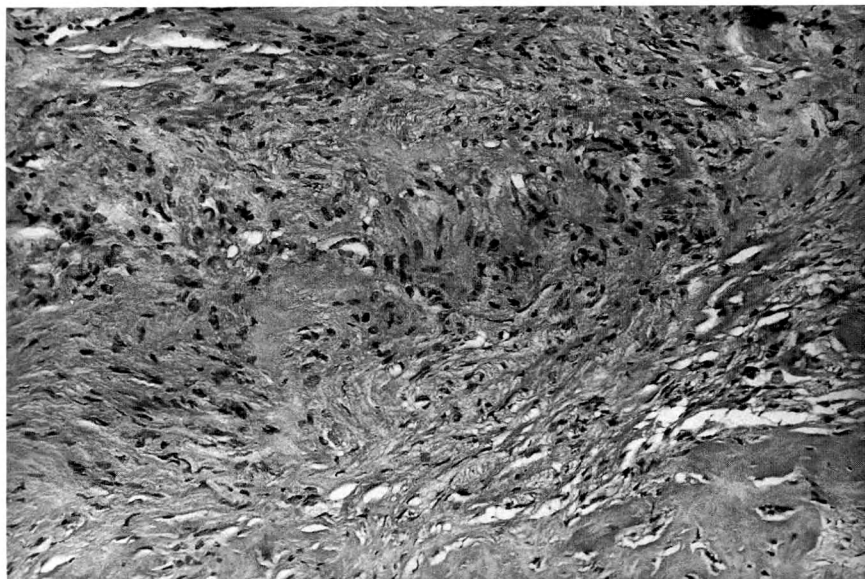


Fig 1. (Case 1) Light micrograph illustrating palisading spindle cell proliferation amid fibrillary stroma—so-called Antoni type A pattern (H & E, original $\times 200$).

posterior fossa dye study in the presence of tests suggesting a retrocochlear lesion...^{1(p459)} All of the authors of the early case reports failed to identify peripheral schwannomas preoperatively, but rather, discovered them during labyrinth-destructive procedures used to treat Meniere's disease. Miyamoto et al¹² reported an isolated ILS that failed demonstration on both tomograms of the IAC and computed tomography (CT) scanning of the head with and without contrast. Huang¹³ similarly reported a normal polytomogram of the IAC in a patient who subsequently underwent a transmastoid labyrinthectomy for presumptive Meniere's disease and was found to have an ILS. It was not until gadolinium-enhanced MRI (Gd-MRI) became more common that an ILS was clearly delineated preoperatively — by Saeed et al⁴ in 1994. Later that same year, Doyle and Brackmann⁵ reported a series of vestibular schwannomas in which 4 isolated ILSs were identified preoperatively with Gd-MRI. The advent of Gd-MRI greatly improved intracranial soft tissue imaging. Gadolinium is a paramagnetic agent that enhances the T1 and T2 relaxation rates of neighboring hydrogen nuclei, thereby shortening the T1 signal and increasing the intensity of T1-weighted scans.¹⁴ This intensifies soft tissue contrast. This form of MRI has been heralded as having unprecedented accuracy.

Herein are reported 2 cases of isolated ILS that both had negative preoperative Gd-MRI evaluations. This is the first time that this entity has been reported.

CASE REPORTS

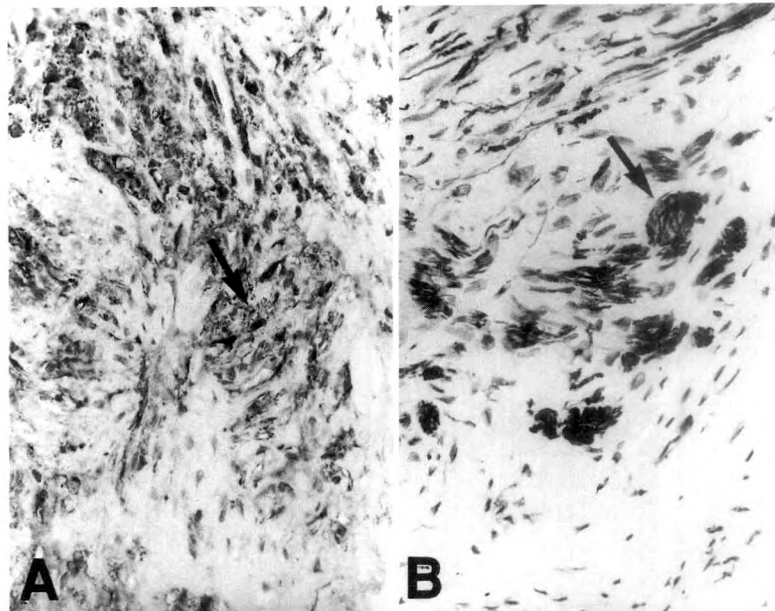
Case 1. A 57-year-old woman presented to the University of Massachusetts Medical Center with a 2-year history of vertigo, tinnitus, and sensorineural hearing

loss (SNHL) in the left ear. Following an acute episode of violent vertigo with nausea and emesis that lasted 2 days, the patient had vertigo spells lasting several hours three to four times each week during the ensuing 2 years. She also noted an acute SNHL in the left ear at the time of the original spell that did not change. She denied prior otologic surgery, otorrhea, or otalgia. She had followed a strict low-sodium diet with diuretic therapy and Valium after receiving a diagnosis of Meniere's disease in the left ear. She had no improvement in her symptoms. A head CT with and without contrast, obtained at her initial presentation 2 years prior, had yielded unremarkable findings.

The patient's past medical history was noncontributory. Findings on physical examination were unremarkable, except for a Weber test lateralizing to the right. On inspection, both ears were normal. No fistula sign was present. An audiogram demonstrated hearing in the right ear that was normal up to 4,000 to 8,000 Hz, where a mild high-frequency SNHL was present. The pure tone examination of the left ear demonstrated profound SNHL. Electronystagmography showed a mild canal paresis on the left consistent with a peripheral vestibular defect. Gadolinium-enhanced MRI with 3-mm sections yielded unremarkable findings. Serology for autoimmune inner ear disease and syphilis was negative.

The patient was judged to have intractable Meniere's disease on the left and brought to the operating room for labyrinthectomy. Upon opening of the labyrinth, soft tissue was noted in the crus commune and ampulla of the posterior semicircular canal, with extension to the vestibule. The tumor mass measured 3 $\times$ 3 mm on total removal. The surgery was completed and the patient recovered uneventfully. At 6-month follow-up, she continued to do well, with no further episodes of ver-

Fig 2. (Case 1) Positive immunohistochemical staining (arrows) with A) anti-S-100 protein and B) anti-neurofilament protein (original $\times 400$).



tigo. The pathologic findings were consistent with a schwannoma (Fig 1).

Case 2. A 43-year-old man suffered head trauma 6 months prior to presentation to The University of Iowa Hospitals and Clinics. He did not lose consciousness at the time of injury. There was no history of otorrhea. Approximately 4 hours after the injury, he noted violent vertigo that lasted for several days. On the third day after the injury, he noted a sudden hearing loss on the right. Since the time of injury, he had noted intermittent episodes of spontaneous, whirling vertigo every 2 to 3 days, lasting several hours each episode. Results of cervical spine radiography and head CT with contrast performed at a local emergency department were reportedly normal. Subsequent audiologic examinations by a local otolaryngologist were significant for profound SNHL on the right.

The patient's past medical history was noncontributory. Findings on physical examination were unremarkable, except for a Weber test that lateralized to the left ear. On inspection, both ears were normal. There was no positive fistula sign. Pure tone audiologic examination demonstrated a mild to moderate SNHL at 250 Hz that sloped upward to normal in the middle frequencies and then sloped back to a profound high-frequency SNHL in the left ear. The speech reception threshold was 15 dB, with a discrimination score of 100%. The contralateral pure tone audiologic examination demonstrated a profound SNHL on the right with no response. Electronystagmography showed a moderate canal paresis on the right. Gadolinium-enhanced MRI with 3-mm cuts yielded unremarkable findings.

The patient was thought to have a traumatic perilymphatic fistula on the right. Although traumatic en-

dolymphatic hydrops could not be ruled out, the time course was felt to be too rapid. Given his profound deafness, a right-sided labyrinthectomy was recommended. Upon surgical exploration, a 4 $\times$ 3-mm soft tissue mass from the ampullated end of the posterior semicircular canal, extending through the vestibule to the superior semicircular canal, was identified. The operation was completed without incident. On 6-month follow-up, the patient was doing well, with complete resolution of vertigo spells. The pathologic findings were consistent with a schwannoma.

DISCUSSION

There are at least 32 cases of isolated ILSs reported in the English-language literature that were discovered at either operation or autopsy.^{2,13,15-17} Among these cases, Gd-MRI was performed in 8 patients preoperatively, and all cases were successfully detected.⁴⁻⁶ Gadolinium-enhanced MRI has undisputably emerged as the best modality for evaluating schwannomas, detecting lesions as small as 3 mm.¹⁸

The exact mechanism of Gd enhancement of neural structures is unknown. However, increased vascularity secondary to nerve edema — which may be present adjacent to tumors — is thought to play a possible role.¹⁹ The absence of Gd enhancement in the 2 patients presented in this review is surprising. Pathologic diagnoses of schwannoma were confirmed by immunohistochemical markers. With positive staining using monoclonal antibodies to S-100 protein and neurofilament protein, the diagnosis of schwannoma can be supported. S-100 protein occurs in many cell types, including Schwann cells. Neurofilament protein is characteristic of neuronal cells. The positive staining with both these markers is consistent with proliferation of an ectopic

nerve (Fig 2).

Although both tumors were less than 5 mm in size, they should have been identified by Gd-MRI with 3-mm cuts. It is unlikely that these tumors represent an enhancement-resistant form of schwannoma, but nevertheless, an explanation for the absence of enhancement is lacking. Retrospective review of the Gd-MRI by two neurotologists and two neuroradiologists failed to reveal enhancing tumor in either of these cases. The most likely explanation is that these tumors were just shy of enhancement with the standard concentrations of Gd and the particular angle of the MRI cuts, and so were invisible. However, there are at least 8 reported cases of isolated ILSs detected by Gd-MRI preoperatively, of which 5 ILSs were remarkably small.⁴⁻⁶ The 2 cases presented in this report come from separate institutions in which similar MRI scanners were used, and therefore, repetitive technical or radiographic error is unlikely.

This review demonstrates that there are certain isolated ILSs that despite Gd-MRI may be missed on complete workup. This conclusion would imply that a small population of patients with the diagnosis of Meniere's disease might actually have an isolated ILS as the cause of their symptoms. These isolated ILSs may never be revealed unless these patients happen to undergo destructive labyrinthine surgery for intractable Meniere's disease. Only in situations of absent ipsilateral hearing in which transmastoid labyrinthectomy is utilized will isolated ILSs be serendipitously discovered. Neither transcanal labyrinthectomy, endolymphatic shunt operation, labyrinth-sparing vestibular nerve section, nor instillation of intratympanic gentamicin will reveal the true underlying disease. Thus, the statement of Babin and Harker¹ is still valid today, but with this revision: small isolated ILSs remind us that in the presence of a history suggestive of retrocochlear disease, even a negative Gd-MRI scan does not rule out such a lesion.

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MIDDLE EAR GAS COMPOSITION IN PATHOLOGIC CONDITIONS: MASS SPECTROMETRY IN OTITIS MEDIA WITH EFFUSION AND ATELECTASIS

LEIF HERGILS, MD, PhD

BENGT MAGNUSON, MD, PhD

LINKÖPING, SWEDEN

It is generally assumed that the gas enclosed in the middle ear and mastoid air cell system is subject to continuous absorption, and that partial pressures for oxygen and carbon dioxide equal those of "mixed venous blood." We previously determined the concentrations in 10 adults with healthy ears by means of mass spectrometry. The results showed that the value for oxygen was in the expected range, but that the value for carbon dioxide was higher than in "mixed venous blood." The aim of the present investigation was to measure the gas concentrations with the same technique in diseased ears, to see if there are any differences in middle ear gases between healthy and diseased ears. In terms of group means, the present measurements in 13 ears of children with otitis media with effusion or atelectasis did not differ from the values previously obtained for healthy ears of adults for any of the gases.

KEY WORDS — mass spectrometry, middle ear gas composition, on-line measurements, pathology.

INTRODUCTION

We have studied middle ear pressure regulation for a long time, concerning both pathoetiologic factors in retraction-type middle ear disease^{1,2} and pressure regulation in the normal human middle ear.³⁻⁷ As part of the latter studies, we measured the gas composition in normal adult middle ears using mass spectrometry — the first on-line measurements in humans.⁶

Different mathematical models of gas diffusion in the middle ear have been proposed, and one model, by Ostfeld and Silberberg,⁸ was checked against our previous experimental values.⁷ It is obvious that we need more information on diffusion rates for different gases and on gas composition during different pathologic conditions to understand the dynamics of middle ear pressure regulation. In order to find some of the answers, we performed studies on the gas composition in middle ears of children with otitis media with effusion (OME) or atelectasis. We used the same mass spectrometric technique that we used in our study on normal middle ears.⁶

MATERIALS AND METHODS

Thirty-nine children from 5 to 15 years old with OME and/or deeply retracted tympanic membranes who were scheduled for insertion of transmyringal ventilation tubes were anesthetized with intravenous Diprivan and spontaneous air breathing, without any anesthetic gases. In cases in which pulse oximetry showed a decrease of oxygen saturation to $\leq 90\%$,

oxygen was added while maintaining spontaneous breathing. The ears were examined by otomicroscopy, and in all ears with a remaining gas pocket in the middle ear (even just a small bubble), the gas concentrations were measured with a quadrupole mass spectrometer (Balzers QMG 420, Lichtenstein). The details of this mass spectrometric system and its high accuracy have been published.⁶ After calibration of the measuring system, the external ear canal was continuously flushed with pure helium gas to exclude contamination of the middle ear gas with room air during sampling. A leak of helium into the middle ear makes no difference in this case. The partial pressures of the gas mixture in the middle ear change, but the original proportions of oxygen, carbon dioxide, nitrogen, and argon are not changed by gas diffusion during our short measuring period. In the measurement process, the sum of these gases was set at 100%, excluding the helium signal. Preventing contamination of the sample with room air by using helium in the external ear canal, and then excluding helium in the measurements, provides an important gain in precision. A thin spinal needle was used for puncturing the gas pocket through the tympanic membrane. The mandrin was left in place to throttle the flow and minimize the gas consumption. The needle was connected directly to the mass spectrometer by a Teflon catheter, giving an on-line system with a continuous, unbroken line from the site of measurement (the middle ear cavity) to the analyzer. The needle was first introduced in the external ear canal while check-

From the Departments of Audiology (Hergils) and Otorhinolaryngology (Magnuson), Linköping University Hospital, Linköping, Sweden.

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CORRESPONDENCE — Leif Hergils, MD, PhD, Dept of Audiology, Linköping University Hospital, S-581 85 Linköping, Sweden.

TABLE 1. RELATIVE DRY GAS CONCENTRATIONS IN MIDDLE EARS WITH OME AND ATELECTASIS, COMPARED WITH NORMAL ADULT MIDDLE EARS

	Nitrogen (%)	Oxygen (%)	Argon (%)	Carbon Dioxide (%)
OME	85.77 ± 1.09	5.66 ± 1.17	1.00 ± 0.03	7.57 ± 0.28
Atelectasis	85.74 ± 0.83	5.43 ± 0.69	0.99 ± 0.02	7.83 ± 0.29
Normal	85.81 ± 0.55	5.72 ± 0.52	0.99 ± 0.01	7.47 ± 0.78

Concentrations are mean ± SD.
OME — otitis media with effusion.

ing the presence of a 100% helium milieu. The tympanic membrane was then punctured, and a few seconds later the needle was withdrawn again. Typically, we obtained about 100 consecutive measurements of each gas from the middle ear. The tympanic membrane was then incised with a myringotomy knife, fluid was removed by suction, and finally, a ventilation tube was put in place. In the 39 children, a small gas pocket that could be punctured was present in 25 ears. Good measurements were obtained in a total of 13 ears. The most common cause for exclusion was aspiration of fluid in the puncturing needle, which prevented a free flow of gas, thus making measurement impossible.

RESULTS

The relative concentrations for the different gases are given as group means and standard deviations in Table 1. No significant differences were present between atelectatic ears ($n = 5$) and ears with serous otitis media ($n = 8$): $p > .05$. The results are given as relative concentrations of dry gases, since it was not possible to measure the total pressure in these ears, which sometimes contained just a tiny bubble of gas (in contrast to the study in normal ears). For comparison, the results from our earlier study of healthy middle ears in adults are included. Unpaired two-tailed t -tests showed no significant differences between healthy adult ears and OME or atelectatic ears in children for any of the measured gases ($p > .05$). A few of the children received oxygen while breathing spontaneously, but this did not seem to influence the gas composition in the middle ear cavity (Table 2). The children had a similar composition of the middle ear gas mixture except for one case: a 6-year-old girl had a fluid-filled atelectatic ear with a very small gas bubble in the protympanum. The measurement was successful and showed an extremely low concentration for oxygen (2.88%) with a reciprocal increase of nitrogen (88.27%).

DISCUSSION

Other investigators have used gas chromatography^{9,10} or gas electrodes^{11,12} to study the gas composition in healthy and diseased human ears. These

TABLE 2. RELATIVE DRY GAS CONCENTRATIONS IN MIDDLE EARS OF SUBJECTS WHO DID OR DID NOT RECEIVE OXYGEN DURING ANESTHESIA

	Nitrogen (%)	Oxygen (%)	Argon (%)	Carbon Dioxide (%)
No oxygen ($n = 10$)	85.82 ± 1.09	5.52 ± 1.11	1.01 ± 0.03	7.66 ± 0.33
Oxygen ($n = 3$)	85.55 ± 0.26	5.74 ± 0.45	0.98 ± 0.02	7.72 ± 0.21

Concentrations are mean ± SD.

methods involved aspiration of middle ear gas in a microliter syringe or glass capillaries with puncture cannula, and then injecting the gas in the measuring apparatus. This transfer carries the inevitable risk of contaminating the minute gas sample with room air.

There are some earlier studies on middle ear gas concentrations in OME patients. The measurements of Guillemin et al⁹ in a group of healthy and diseased ears showed much lower carbon dioxide concentrations ($n = 16$, $5.6\% \pm 1.54\%$) and much higher oxygen concentrations ($10.08\% \pm 2.39\%$) than in our study, which might reflect admixture with room air to the samples. One patient had middle ear effusion, but the partial pressures for oxygen, carbon dioxide, and nitrogen apparently did not differ from those in patients with healthy ears. Ingelstedt et al¹¹ studied partial pressures of oxygen and carbon dioxide in patients with OME ($n = 7$). They analyzed the partial pressures of gases dissolved in middle ear effusion using a gas electrode technique. Their oxygen values ($5.75\% \pm 0.97\%$) did not differ significantly from our measurements of middle ear gas in OME patients, but the carbon dioxide values were significantly higher than ours ($8.11\% \pm 0.62\%$; $p < .05$). Our gas samples were taken from the middle ear cavity, while Ingelstedt et al took their effusion samples from the air cell system. The perfusion, and the local metabolism in the mucosa might differ in those different locations, and this difference might explain differences in carbon dioxide pressure.

Our results are the first on-line measurements with reliable protection against contamination in human ears with pathologic changes. We used the same technique we used for studying normal human ears,⁶ which makes possible a valid comparison between the gas compositions under the different conditions. Another mass spectrometric technique has been used by others for on-line middle ear gas studies in guinea pigs.^{13,14} Their technique was essentially the same with the exception of a different inlet system. They used a diffusion catheter, while we used a highly throttled respiratory inlet system. The advantage of a diffusion catheter is its substantially lower gas consumption, making measurements of long dynamic

events possible. Advantages of our inlet system are lack of sensitivity to actual changes of temperature, much shorter response time, lack of risk of contamination with room air, easier sterilization and calibration, and easier introduction of the probe in the human middle ear.

Our findings are consistent both with the "sniffing" theory² and with the classic "hydrops ex vacuo" theory. The exceptionally low oxygen concentration found in 1 of our subjects might be due to oxygen consumption by bacteria present in the effusion. Our finding that there was no difference in middle ear gas

composition between normal ears and ears with OME or atelectasis does not mean that there was no difference in total pressure. Further, the finding of no measurable differences in the steady-state compositions of the gas mixtures does not mean that the diffusion process is identical. Even though the end result was the same in the healthy and diseased ears investigated, the gas exchange by diffusion over the middle ear mucosa may be different in healthy and diseased ears. Our studies so far are short-term measurements that give only an instant picture, and there is a need for long-term measurements of changes over time regarding middle ear gas concentrations in humans.

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CLINICAL OBSERVATIONS ON ACUTE LOW-TONE SENSORINEURAL HEARING LOSS

SURVEY AND ANALYSIS OF 137 PATIENTS

SHUN-ICHI IMAMURA, MD

MAYUMI IMAMURA, MD

IZURU NOZAWA, MD

YOSHIHIKO MURAKAMI, MD

YAMANASHI, JAPAN

We retrospectively analyzed the clinical records of 137 patients who were treated at our clinic for acute low-tone sensorineural hearing loss of unknown cause over a period of 8 years. The analyses of the clinical records indicated the following clinical characteristics: female preponderance; peak incidence during the fourth decade of life; frequent accompanying tinnitus, sensation of ear fullness, and/or autophony; suspicion of bilateral involvement; and association with autonomic imbalance. The hearing in most of the patients completely recovered, but some selected patients experienced fluctuating hearing loss or progression to Meniere's disease. Our results suggest that a subgroup of patients with severe initial hearing loss should be carefully followed up, although it is difficult to predict the outcome of this disorder.

KEY WORDS — autonomic imbalance, bilaterality, low-tone hearing loss, statistical survey.

INTRODUCTION

Patients who complain of low-tone hearing loss of acute onset are relatively commonly encountered in daily clinical practice. They frequently complain of various symptoms including autophony, low-pitched tinnitus, sensation of fullness in the ear, and a mild dizzy sensation accompanied by hearing impairment. The pure tone audiogram in these patients indicates sensorineural hearing loss restricted to the low frequency range.

The first clinical review of patients with low-tone sensorineural hearing loss was that reported by Abe¹ in 1982. Unlike in idiopathic sudden sensorineural hearing loss, the hearing impairment never progresses to involve the high-frequency range. Unlike Meniere's disease, the disorder shows no initial association with vertigo or nystagmus. Usually, these patients do not have a severe hearing loss, and sometimes the symptoms are neither stable nor obvious. Moreover, the hearing of these patients frequently recovers after a relatively short period, even without treatment. Therefore, little attention has been given to this disorder by either clinicians or the patients themselves. Although most of the patients complain of only a mild subjective hearing loss, it is reported that some of them have fluctuation of hearing and/or subsequent progression to Meniere's disease.²⁻⁵

In previous reports, this condition has been interpreted as an independent disease entity and referred to as low-tone sudden deafness^{1,3,5} or acute low-tone sen-

sorineural hearing loss (ALHL).^{2,4} We retrospectively investigated the clinical features of 137 patients with ALHL in this study. These clinical features and a possible mechanism of the pathogenesis of ALHL are described herein.

PATIENTS AND CRITERIA

We retrospectively analyzed the patient records at our outpatient clinic for 137 patients who fulfilled the criteria given below. Although various diagnostic criteria of ALHL have been described in previous reports,¹⁻⁵ they have not as yet been generally established or accepted. Our diagnostic criteria for ALHL are as follows: 1) the hearing loss is purely sensorineural in nature, while the tympanometry (model RS20, RION) shows normal type A and normal tympanic membrane findings; 2) on the pure tone audiogram (model AA61BN, RION), the average hearing loss at the three low frequencies (125, 250, and 500 Hz) is 30 dB or more, and the average hearing loss at the three higher frequencies (2, 4, and 8 kHz) is 20 dB or less; and 3) the patients have no known etiologic factors that might cause low-tone sensorineural hearing loss as detected by routine clinical and radiologic examinations. Patients with a past history of episodic vertigo or with spontaneous nystagmus at the initial examination were strictly excluded.

Also not yet established are the criteria for evaluating the outcome in ALHL. Our patients were evaluated according to the following prognostic criteria,

From the Department of Otolaryngology, Yamanashi Medical University, Yamanashi, Japan.

CORRESPONDENCE — Shun-ichi Imamura, MD, Dept of Otolaryngology, Yamanashi Medical University, 1110 Shimokato, Tamaho-cho, Nakakoma-gun, Yamanashi, Japan, 409-38.

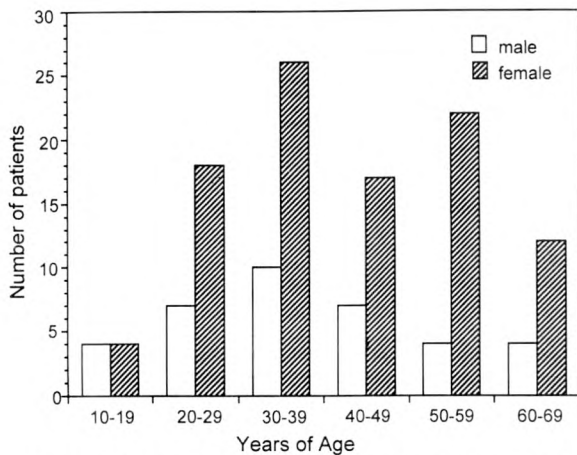


Fig 1. Age distribution of patients with acute low-tone sensorineural hearing loss (ALHL). In both male and female patients, peak incidence was in thirties.

which were produced as a model of prognostic criteria for idiopathic sudden deafness by the Otological Society of Japan: complete recovery, in which hearing at low frequencies (125, 250, and 500 Hz) returns to within 20 dB of normal; partial recovery, in which the average loss at the low frequencies at the final follow-up is improved by 10 dB or more compared to the initial audiogram; unchanged, in which the average loss at the low frequencies at the final follow-up is within 10 dB of that at the initial audiogram; progression, in which the final average loss at the low frequencies exceeds by more than 10 dB that at the initial audiogram; and fluctuation, in which an average loss at the low frequencies of 30 dB or more is seen again following complete or partial recovery. In this study, the outcome of the patients who were followed up for more than 3 weeks after the first examination was evaluated. An unpaired Student's *t*-test was used to evaluate the results, and a *p* value of less than .05 was accepted as significant.

At our clinic, the patients with sensorineural hearing loss were routinely examined by short increment sensitivity index (SISI), Bekesy tracing (model AA61B, RION), and the Metz test (model RS20, RION) in the testing chamber. The vestibular (positional and positioning nystagmus and caloric test) and autonomic nerve functions (blood pressure and Schellong test) were also tested. However, all of these examinations could not routinely be administered to the ALHL patients. Some of them were not willing to undergo further detailed examinations because their complaints were relatively mild. The Schellong test was used as an orthostatic test for screening autonomic nervous system functions. The method of this test as used in this study is described briefly as follows. After 10 minutes in the supine position, resting blood pressure and pulse rate were measured; and after 1, 5, and 10 minutes in the stand-

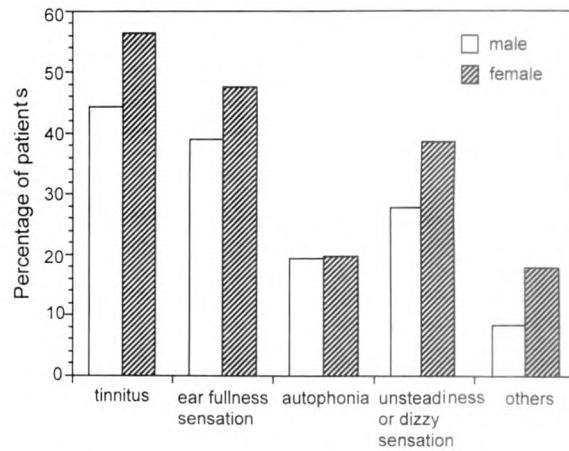


Fig 2. Percentages of patients with concomitant symptoms. Tinnitus, ear fullness sensation, and unsteadiness frequently accompanied ALHL, especially in female patients.

ing position, blood pressure and pulse rate were measured again. Sixty-seven of the ALHL patients and 90 healthy volunteers (the control group, who had never experienced hearing impairment or vertigo) underwent the orthostatic test. The control group was composed of 42 males from 14 to 75 years of age (mean age 37.5 years) and 48 females from 16 to 72 years of age (mean age 36.9 years). In the evaluation of the orthostatic test results, a positive test result was defined as a 21 mm Hg or greater decrease in the systolic pressure, a 16 mm Hg or greater decrease in the pulse pressure, or a 21 beats per minute or more increase in the pulse rate.

RESULTS

Patient Status. There were 101 female (74%) and 36 male (26%) patients; approximately three fourths of the patients were female. Three males and 11 females, approximately 10% of patients, experienced bilateral hearing impairment. There was minimal difference of age distribution between males and females, and the peak incidence by age in both was the fourth decade of life (Fig 1). The mean age of all the patients was 40.6 years (males 38.9 years, and females 40.8 years).

Clinical Symptoms. The symptoms of the ALHL patients varied widely (Fig 2), and the chief complaint of the ALHL patients was not always hearing impairment. Over half of the patients had tinnitus, which often took the form of a low-pitched, motor-like tone. A sensation of fullness in the ear and autophony were also frequently observed. Nearly one third of the patients had unsteadiness or a dizzy sensation immediately after rising. In particular, female patients often complained of vague, mild dull headache, stiff shoulders, fatigue, and so on, which are similar to the symptoms of autonomic imbalance

AVERAGE HEARING LEVELS IN PATIENTS WITH UNILATERAL INVOLVEMENT (N = 123)

Patients	Ears	125 Hz	250 Hz	500 Hz	1,000 Hz	2,000 Hz	4,000 Hz	8,000 Hz	Low Frequencies*	High Frequencies†
Male	Affected	43.6	45.8	34.5	15.5	11.8	16.5	21.4	41.3	16.6 ^b
	Unaffected	20.0	18.8	14.5	10.5	13.2	15.3	24.4	17.8 ^a	17.6 ^c
Female	Affected	45.0	45.9	34.7	15.4	13.4	13.8	23.0	41.8	16.8 ^e
	Unaffected	22.2	21.3	16.2	9.5	11.3	12.5	19.0	19.9 ^d	14.3 ^f

Data are in decibels. For d versus f, $p < .01$. No significance for a versus c, b versus c, or e versus f.
 *Average of levels at 125, 250, and 500 Hz.
 †Average of levels at 2,000, 4,000, and 8,000 Hz.

patients.

Audiometric Pattern. The mean values of the initial average hearing losses at the three low frequencies in the affected ears were 41.3 dB in the male and 41.8 dB in the female patients. The mean values of the average losses at the three high frequencies were 16.6 dB and 16.8 dB, respectively (see Table).

In the unaffected ears in females with unilateral involvement, the loss for the low frequencies was greater than that for the high frequencies, although the average loss for the low frequencies did not reach 30 dB (see Table). This difference between the total losses for high and low frequencies in the unaffected ears was statistically significant (t -test, $p < .01$); even when the contralateral ear was determined to be unaffected, the hearing for low frequencies was somewhat impaired compared to that for high frequencies. Thus, the patients with ALHL appeared to have a tendency for bilateral involvement, especially the females.

Clinical Examination. Eight (11.4%) of 70 patients showed a recruitment phenomenon. A more than 60% positivity on the SISI, a less than 55-dB difference of threshold on the Metz test, or a less than

3-dB amplitude narrowing of the continuous trace on Bekesy audiometry was deemed to imply the presence of a recruitment phenomenon. The incidence of nystagmus on positioning and/or positional stimulation was 9.3% among the 75 patients who underwent these tests, and a reduced caloric response in the affected ear was observed in 11.3% of the 62 patients who underwent a caloric test.

In 32 of the 85 patients whose blood pressure was measured, the blood pressure in the resting position indicated hypotension, with a systolic pressure of 100 mm Hg or less. In the control group of 90 subjects, only 6 had hypotension.

On the Schellong test, 46% of 67 ALHL patients had a positive result, while only 28% of the control group did ($p < .01$).

Outcome. Most of the patients were treated with orally administered drugs, such as vitamin B12 and adenosine triphosphate disodium, at our outpatient clinic. Only a few patients took prednisolone at various dosages. The evaluation of the hearing outcome, performed in 25 males and 82 females including 3 patients with bilateral involvement, revealed com-

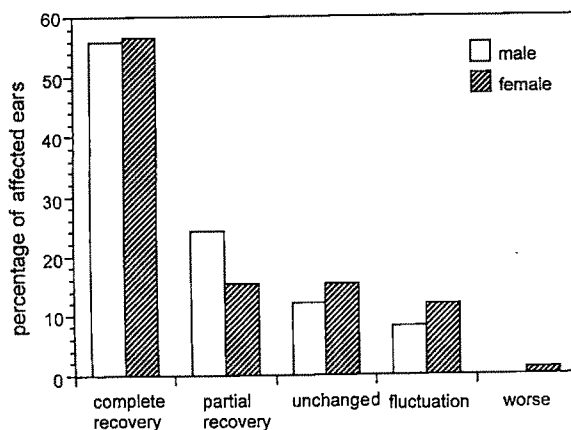


Fig 3. Percentages of affected ears showing various outcomes in ALHL patients. More than half of patients showed complete recovery of hearing. Better than partial recovery was seen in 80% of males and 70% of females. However, 10% of patients experienced hearing fluctuation.

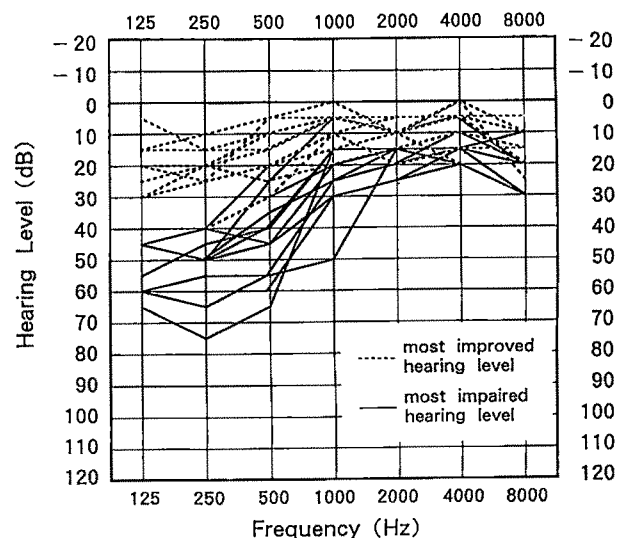


Fig 4. Superimposed pure tone audiograms from patients with fluctuating hearing ($n = 12$).

plete recovery in more than half of them. More than 70% of the female patients and 80% of the male patients showed complete or partial hearing recovery (Fig 3). However, 2 males and 10 females experienced fluctuating hearing during the course of the follow-up period. The patients who experienced fluctuating hearing were widely distributed in age (from 27 to 66 years), but their mean age (42.3 years) was not markedly different from that of all the patients. There were no prominent features identified by clinical examination in this subgroup. Figure 4 shows superimposed audiograms of the patients with fluctuating hearing. The range of hearing fluctuation in the average losses at the low frequencies was extremely wide: 18.3 to 51.7 dB (mean of 29.2 dB).

DISCUSSION

Our analyses of these patients with ALHL indicated a unique collection of several clinical features, including a quite evident female preponderance, a peak age of incidence in the thirties (younger than that of sudden hearing loss⁶⁻⁹), and tinnitus, sensation of ear fullness, and autophony frequently accompanied by hearing impairment. Although the hearing recovered quickly in most patients, some of them experienced recurrence of the hearing disturbance. These features are not different from those given in previous reports,¹⁻⁴ but our analysis of the clinical data of ALHL patients did reveal two additional, novel clinical characteristics.

First, more than 10% were affected bilaterally. This is relatively higher than the incidence in sudden deafness.⁷⁻¹⁰ In the unaffected ears of female patients with unilateral involvement, the average loss for the low frequencies was greater than that for the high frequencies, though the loss did not reach the level of the diagnostic criteria for ALHL. The tendency toward bilaterality of the disease might be of some significance in explaining its possible pathogenesis or causation.

Second, the patients with ALHL frequently had positive results on the Schellong (orthostatic) test. Hypotension was also found in more than one third of the patients. Two thirds of patients with a positive Schellong test indicated hypotension. The ALHL patients seemed to have an abnormal systemic condition suggesting an underlying autonomic imbalance and/or insufficient blood circulation. Of interest, in some selected patients, medication and/or physical treatment for hypotension or autonomic imbalance was followed by improvement in their condition and hearing.

It is supposed that a cause similar to that of idiopathic sudden deafness may be related to this disorder. Our ALHL patients showed a higher incidence of complete

recovery than is reported for idiopathic sudden deafness patients.⁷⁻¹⁰ However, it is well known that patients with idiopathic sudden deafness who have an audiometric pattern of loss involving the low frequencies often have a better outcome than those with other audiometric patterns.⁷⁻⁹ It is supposed that there may be some overlap between ALHL and idiopathic sudden deafness that involves impairment at predominantly low frequencies.

On the other hand, a few patients have experienced hearing fluctuation and vertiginous episodes. There may be a possibility, as pointed out by several authors, that ALHL overlaps with an early stage of atypical Meniere's disease or endolymphatic hydrops of the cochlea without vertigo, as suggested by Williams et al.¹¹ The results of an electrocochleographic study suggested that the pathogenesis of ALHL is similar to that for endolymphatic hydrops.¹² In fact, it has been established by Tonndorf¹³ that endolymphatic hydrops of the cochlea can produce a low-frequency hearing loss.

As noted above, our ALHL patients frequently showed abnormality on the orthostatic test, suggesting that some of the patients had autonomic imbalance as an underlying condition. A clinical study using ultrasonic rheography revealed that vertiginous patients with vascular hypotension and/or orthostatic hypotension tend to have insufficiency of vertebral blood flow.¹⁴ On the other hand, experimental sympathetic stimulation has caused dysregulation of cerebral and cochlear blood flow.¹⁵⁻¹⁷ We are inclined to consider that some transient or reversible circulatory disturbance in the cochlea caused by autonomic nerve dysfunction might be related to the development of ALHL with a good prognosis, although these mechanisms have not yet been confirmed. The hearing improvement and relief of otologic symptoms often achieved by treatment for hypotension and/or orthostatic dysfunction may support this hypothesis to some extent. In addition, prominent bilaterality of ALHL might also support this supposition.

Our retrospective investigation of patients who had ALHL of unknown cause revealed that such patients presented a unique constellation of clinical features. Most of the patients had only relatively mild and transient complaints and achieved prompt complete or partial hearing recovery. Therefore, ALHL can be treated as an independent disease entity that is a milder disorder than idiopathic sudden sensorineural hearing loss or Meniere's disease. In some selected patients, however, fluctuating hearing and/or vertiginous episodes developed. It is thus desirable to identify this subgroup of patients at initial examination.

Our survey and analyses failed to reveal any characteristic clinical features that are exclusively found in the patients who have recurrence of hearing loss or development of vertiginous episodes, except that in these patients there was the tendency for the initial average hearing loss at low frequencies to be greater

than that of the patients with good outcome. It may be necessary for the patients who have relatively severe low-tone hearing loss at initial examination to be followed up more carefully, although at present it is not possible to predict the outcome of patients with ALHL.

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TWENTIETH ANNUAL MEETING OF THE BÁRÁNY SOCIETY

The Twentieth Annual Meeting of the Bárány Society will be held in Würzburg, Germany, September 20-23, 1998. For more information, contact the Local Organizing Committee, Institute of the 4-G-F, Kurhausstrasse 12, D 97688 Bad Kissingen, Germany; telephone 0049/971-64832; fax 0049/ 971-68637.

BACTERIAL FLORA OF STETHOSCOPES' EARPIECES AND OTITIS EXTERNA

ITZHAK BROOK, MD, MSC
WASHINGTON, DC

External otitis caused by *Staphylococcus aureus* was observed in a nurse after extensive use of a stethoscope. The infection recurred and a similar organism was isolated from the stethoscope's earpiece. The infection did not recur after the earpiece was cleansed after each use. In a prospective study, the bacterial flora of 35 earpieces was evaluated. Fifty-three isolates, 36 aerobic or facultative and 17 anaerobic, were recovered. The number of organisms per earpiece ranged from 14 to 204 (average 92 ± 17). The predominant isolates were *Staphylococcus epidermidis* (16 isolates), *Propionibacterium acnes* (12), and *S aureus* (7). The study demonstrates the colonization of the stethoscope's earpiece with microorganisms that possess the potential for causing nosocomial infection.

KEY WORDS — otitis externa, *Propionibacterium acnes*, *Staphylococcus aureus*, stethoscopes.

INTRODUCTION

The diaphragms of stethoscopes are known to be colonized with microorganisms and serve as a potential source of nosocomial infection.^{1,2} Cleansing of the diaphragm with alcohol has been shown to reduce the colonization rate of the stethoscope with coagulase-negative staphylococci.¹ However, the potential importance of the stethoscope's earpiece as a source of nosocomial infection, as well as a cause of external ear canal infection, has not been extensively evaluated.

This study was undertaken after encountering a case of external otitis that developed in conjunction with the use of a stethoscope. The bacterial flora of earpieces was studied in stethoscopes used in a hospital.

CASE REPORT

A 42-year-old female nurse was examined for bilateral external otitis of 1 week's duration. She had no previous ear infections and was in good health. Two days before onset of the otitis, she used her stethoscope extensively to monitor blood pressure on a seriously ill patient. She noticed pain and redness in her ear canals about 3 hours after finishing work that day. Otologic examination revealed diffuse redness, swelling, and purulent discharge from both external auditory canals and earlobes. Bacterial culture yielded *Staphylococcus aureus* that was resistant to methicillin sodium. Topical therapy with 2% acetic acid and 1% mupirocin ointment was given for 10 days. The patient experienced resolution of her symptoms. However, 10 days after the conclusion of therapy, her symptoms recurred. Culture of the ear canal revealed *S aureus* resistant to methicillin. Culture of her stethoscope's earpieces revealed a similar organism. She was instructed to clean

her stethoscope with a 70% isopropyl alcohol swab after each use, and to refrain from lending it to other nurses. Therapy with topical 2% acetic acid and 1% mupirocin ointment was given again for 10 days. The patient experienced complete resolution of the infection, and no recurrence in a 12-month follow-up.

MATERIALS AND METHODS

Thirty-five stethoscopes used individually by nurses in a pediatric department were studied. All bacterial specimens were collected within 5 to 15 minutes of their use and were obtained with a sterile cotton swab moistened with sterile saline. The entire external area of each of the right earpieces was thoroughly swabbed. All specimens were processed immediately. They were shaken vigorously inside a test tube containing 1 mL of sterile saline. Specimens were processed for aerobic and anaerobic bacteria.

Quantitative bacterial analyses were performed by plating serial dilutions of the saline solution. Isolations for aerobic bacteria were made on brain-heart infusion agar. Sheep's blood agar plates enriched with vitamin K1 and hemin were used for isolation of anaerobic bacteria. Aerobic and anaerobic bacteria were identified according to published methods.^{3,4}

RESULTS

Bacteria were recovered from all earpieces. Fifty-three organisms were recovered — 36 aerobic or facultative, and 17 anaerobic isolates — accounting for 1.0 aerobic or facultative bacteria and 0.5 anaerobic bacteria per specimen (see Table). The number of organisms per earpiece ranged from 14 to 204 (average 92 ± 17). There were no differences in the rate of recovery of

From the Department of Pediatrics, Georgetown University School of Medicine, Washington, DC. The opinions and assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.
CORRESPONDENCE — Itzhak Brook, MD, MSc, PO Box 70412, Chevy Chase, MD 20813-0412.

BACTERIA ISOLATED FROM RIGHT EARPIECE OF
35 STETHOSCOPES

Bacteria	No. of Isolates
Aerobes and facultatives	
<i>Staphylococcus epidermidis</i>	16
<i>Staphylococcus aureus</i>	7
α -Hemolytic streptococci	4
<i>Enterococcus faecalis</i>	2
<i>Bacillus</i> sp	4
<i>Escherichia coli</i>	1
<i>Pseudomonas aeruginosa</i>	2
Subtotal	36
Anaerobes	
<i>Peptostreptococcus</i> sp	5
<i>Propionibacterium acnes</i>	12
Subtotal	17
Total bacteria	53

various organisms. Aerobic bacteria only were isolated in 21 specimens (60%), anaerobic bacteria only in 3 (9%), and mixed aerobic and anaerobic isolates in 11 (31%). The predominant isolated bacteria were *Staphylococcus epidermidis* (16 isolates), *Propionibacterium acnes* (12), *S aureus* (7), α -hemolytic streptococci (4), and *Bacillus* sp (4).

In 20 (57%) instances, only 1 isolate was recovered. In the remaining 15 (43%) cases, where mixed flora were present, the number of isolates ranged between 2 and 4 per specimen.

DISCUSSION

This study illustrates the colonization of stethoscope earpieces with microorganisms. The origin of these organisms may be the ear canal bacterial flora of the stethoscope's user. The bacteria isolated were similar in type and proportion to those found as the normal flora of the ear canal⁵ and are generally noninvasive. However, as illustrated in the case presented in this report, some of these organisms can induce external ear infection.

Even though organisms such as *S epidermidis* are not known to cause external otitis, they are one of the main causes of nosocomial infection, including infection in low-birth weight infants on the neonatal unit,⁶ and can cause vascular catheter-associated infection.⁷ Other organisms, such as *S aureus* and *Enterococcus* spp, are capable of causing serious infections and have developed resistance to antimicrobial agents.⁷

Previous studies highlighted the importance of the stethoscope's diaphragm as a potential source of transmission of microorganisms in a hospital setting.⁶ However, the data in this report also illustrate the importance of the earpiece section of the stethoscope as a likely cause of nosocomial and external ear infections. Further studies are warranted to explore the correlation of stethoscope's earpieces and microorganisms' nosocomial spread. However, these data suggest the need to cleanse the stethoscope diaphragm as well as the earpieces in an effort to reduce the nosocomial transmission of organisms.

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AGING RAT VESTIBULAR GANGLION: II. QUANTITATIVE ELECTRON MICROSCOPIC EVALUATION

MICHAEL J. LYON, PHD
SYRACUSE, NEW YORK

JEFFREY M. KING, MD
BINGHAMTON, NEW YORK

This laboratory has shown that age-related vestibular ganglion cell loss does not occur in the Wistar rat as it does in humans. However, in that study, intracellular changes were evident. The purpose of the present study was to quantitate some of these changes. The volume densities of mitochondria, rough endoplasmic reticulum (RER), Golgi apparatus, and aging pigment, as well as the diameter of the vestibular ganglion cells, of young (3 to 5 months) and old (24 to 31 months) female Wistar rats were determined by electron microscopy and stereological techniques. The data show a significant decrease in the volume densities of mitochondria (11.4%), Golgi apparatus (8.1%), and RER (8.9%), a significant increase in aging pigment (327%), and no change in mean profile diameter. These results suggest a decreased capacity for oxidative metabolism and protein synthesis that may reflect a decrease in the number of hair cells innervated by each ganglion cell and/or in the number of central connections. In either case, these findings suggest impaired metabolic and functional capabilities.

KEY WORDS — aging, labyrinth, rat, Scarpa's ganglion, vestibule.

INTRODUCTION

Aging has been described as the progressive deterioration and/or accumulation of changes leading to the functional decline of a variety of biologic systems. Among these is the control of balance. While control of equilibrium entails the coordination of several systems (visual, sensorimotor, vestibular), and deterioration in any one could lead to dysfunction, the vestibular system undoubtedly plays a major role. The incidence of dizziness and falls among geriatric patients is high, and risks of severe injury or even death escalate with increasing age.^{1,2} Since the geriatric population will continue to rise over the next several decades, it is increasingly important to identify the pathophysiologic mechanisms of vestibular aging with the hope of directing treatment and/or therapy.

Studies of human vestibular aging have demonstrated a number of degenerative changes in the vestibular neuroepithelium, such as hair cell loss, the accumulation of lipofuscin or aging pigment, increased ciliary fragility and subepithelial cysts,³⁻⁷ and age-related decreases in the number of vestibular nerve fibers and ganglion cells.⁸⁻¹⁰ Rodent animal models have been used in the hope of elucidating the processes involved in these degenerative changes and have been shown to undergo some of the same degenerative changes, that is, degeneration within

the vestibular neuroepithelium, including hair cell loss, accumulation of aging pigment, and subepithelial cysts.^{3,7,11,12} But these degenerative processes do not progress to the point of ganglion cell or nerve fiber loss.^{13,14} It is likely that at least some of the basic mechanisms responsible for age-related change (those not directly related to time itself) are common to all mammals.

In a light microscopic study, this laboratory reported that while there was no ganglion cell loss,¹³ there was a dramatic increase in the aging pigment in the ganglion cells of old animals. On this basis, it was postulated that other intracellular changes were likely to be occurring that could not be detected at the light microscopic level. With electron microscopy and stereological analysis, the goal of the present study was to quantitate age-related changes in the volume densities of mitochondria, rough endoplasmic reticulum (RER), Golgi apparatus, and/or aging pigment. The data indicate that there are significant changes in all of these variables.

MATERIALS AND METHODS

The female Wistar rats used in this study (obtained from the Gerontology Research Center, Baltimore, Md) were divided into two age groups; young (3 to 5 months; 214 to 342 g; n = 6) and old (24 to 31 months; 228 to 486 g; n = 9). These animals were used in a

From the Department of Otolaryngology and Communication Sciences and the Department of Anatomy and Cell Biology, State University of New York Health Science Center, Syracuse (Lyon), and the Department of Otolaryngology, State University of New York Health Science Center, Clinical Campus, Binghamton (King), New York. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the State University of New York Health Science Center, Syracuse. Presented in part at the Association for Research in Otolaryngology Midwinter Meeting, Clearwater Beach, Florida, January 31–February 4, 1988. **CORRESPONDENCE** — Michael J. Lyon, PhD, Dept of Otolaryngology and Communication Sciences, SUNY Health Science Center, 750 E Adams St, Syracuse, NY 13210.

VOLUME DENSITIES OF MEASURED ORGANELLES AND MEAN PROFILE DIAMETER OF VESTIBULAR GANGLION CELLS

	Rat	Golgi Apparatus	Rough Endoplasmic Reticulum	Mitochondria	Aging Pigment	Mean Profile Diameter (μm)
Young group	1	0.0424	0.2214	0.1176	0.0127	19.27
	2	0.0450	0.2370	0.1189	0.0158	18.52
	3	0.0373	0.1862	0.1341	0.0161	21.12
	4	0.0388	0.2343	0.1152	0.0214	19.85
	5	0.0406	0.1885	0.1269	0.0206	18.24
	6	0.0475	0.2206	0.1212	0.0172	20.43
Mean		0.0419	0.2147	0.1223	0.0173	19.57
SEM		± 0.0016	± 0.0091	± 0.0029	± 0.0013	± 0.45
Old group	1	0.0418	0.2048	0.1076	0.0438	20.31
	2	0.0349	0.1608	0.0986	0.0497	19.46
	3	0.0329	0.2115	0.1149	0.0299	20.66
	4	0.0395	0.1778	0.1267	0.0716	22.49
	5	0.0362	0.1853	0.1027	0.0714	21.37
	6	0.0396	0.1990	0.1143	0.0572	18.66
	7	0.0418	0.2136	0.1268	0.0598	20.88
	8	0.0391	0.2138	0.0861	0.0614	18.59
	9	0.0405	0.1897	0.0981	0.0648	19.68
Mean		0.0385*	0.1955*	0.1084*	0.0566*	20.23
SEM		± 0.0010	± 0.0062	± 0.0045	± 0.0045	± 0.43

*Statistically significant difference from young group.

previous study.¹³ The mean survival for this strain of rats is 24.6 months.¹⁵ Animals were anesthetized with 3.5% chloral hydrate (1 mL/100 g body weight) and intracardiac perfusion was performed with Ringer's solution, followed by 2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, pH 7.2, at 37°C containing 0.005% calcium chloride. The vestibular ganglion was dissected free, placed in the same fixative for 2 hours, washed in buffer, and post-fixed with buffered 1% osmium tetroxide for 1.5 hours. The specimens were dehydrated and embedded in Durcupan. Serial 5- μm sections were cut and every fifth section was collected on a glass slide. Randomly selected sections were remounted onto plastic blanks and sectioned for electron microscopy. Ultrathin sections were collected onto formvar-coated 1 $\times$ 2-mm slotted copper grids and stained with uranyl acetate and lead citrate.

The sections were scanned for ganglion cell profiles containing a nucleus and nucleolus at 1,000 $\times$ in a JEOL 100-S transmission electron microscope. The specimen stage was moved only in the x-axis until the end of the section was found. At least two photographic fields were skipped in the y direction before moving back across the section in the other direction. This method of sampling made it impossible to sample the same ganglion cell profile twice. When a ganglion cell profile met the criteria, it was photographed at 6,000 $\times$ for montages (final magnification approximately 18,000 $\times$). The volume density determinations were made with stereological point counting tech-

niques.^{16,17} Briefly, an acetate test grid consisting of points spaced at 1-cm intervals was randomly positioned over the entire montage. Test points intersecting mitochondria, RER, Golgi apparatus, and aging pigment were counted. From the sum of all the points hitting the cell and the proportion hitting the organelle of interest, a volume density was calculated. Diameters were measured with a Numonics 1224 x-y digitizing tablet (Numonics Corp, Lansdale, Pa).

The coefficient of error was calculated for each variable by the methods of Gundersen and Jensen¹⁸ for systematic random sample design. It was determined that 20 ganglion cell profiles from each animal would more than meet the criterion of $p < .05$ for each of the variables. Data were compared by means of Student's *t*-test with a probability of $p < .05$ set as the level for rejection of the null hypothesis.

RESULTS

The Table contains individual animal data for all of the measured variables. Data are presented as the mean $\pm$ standard error of the mean. The typical appearance of a vestibular ganglion cell from a young animal is shown in Fig 1A. This should be compared to that of one from an old animal (Fig 1B). Even at this low magnification, the difference in the accumulation of aging pigment is obvious. Qualitatively, there are differences in the appearance of the aging pigment. In the ganglion cells from young animals, the aging pigment's electron density was usually uniform (Fig 1C), while in the old animal group, its

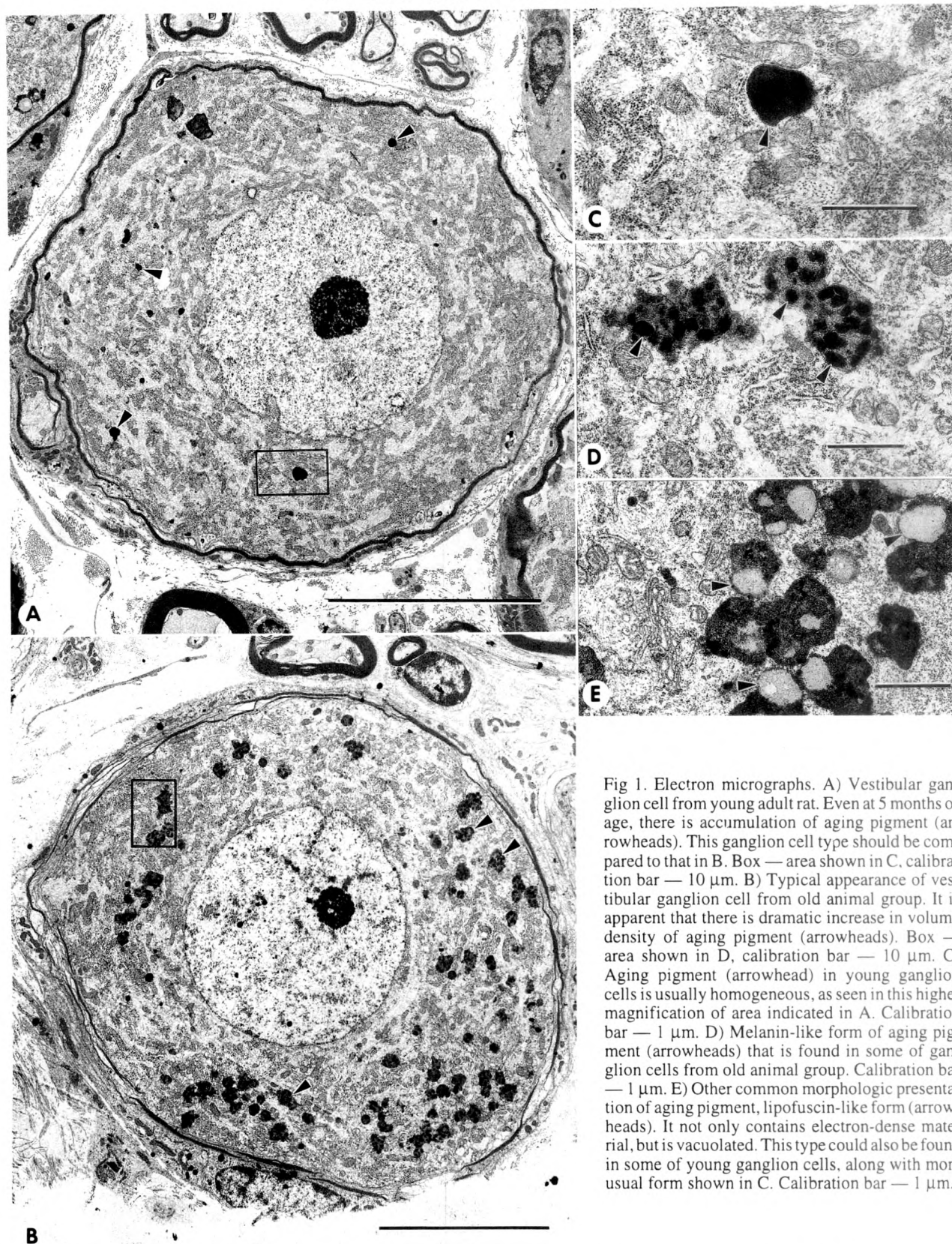


Fig 1. Electron micrographs. A) Vestibular ganglion cell from young adult rat. Even at 5 months of age, there is accumulation of aging pigment (arrowheads). This ganglion cell type should be compared to that in B. Box — area shown in C, calibration bar — 10 μ m. B) Typical appearance of vestibular ganglion cell from old animal group. It is apparent that there is dramatic increase in volume density of aging pigment (arrowheads). Box — area shown in D, calibration bar — 10 μ m. C) Aging pigment (arrowhead) in young ganglion cells is usually homogeneous, as seen in this higher magnification of area indicated in A. Calibration bar — 1 μ m. D) Melanin-like form of aging pigment (arrowheads) that is found in some of ganglion cells from old animal group. Calibration bar — 1 μ m. E) Other common morphologic presentation of aging pigment, lipofuscin-like form (arrowheads). It not only contains electron-dense material, but is vacuolated. This type could also be found in some of young ganglion cells, along with more usual form shown in C. Calibration bar — 1 μ m.

appearance varied. In some cells the aging pigment has a melanin-like appearance (Fig 1D), while in other ganglion cells from the same animal, the pigment resembles lipofuscin (Fig 1E). Quantitative

volume density data indicate that the aging pigment increased by 327%, from a mean of 0.0173 ± 0.0013 at 3 to 5 months of age to 0.0566 ± 0.0045 at 24 to 30 months of age (Fig 2). Old animal data were also

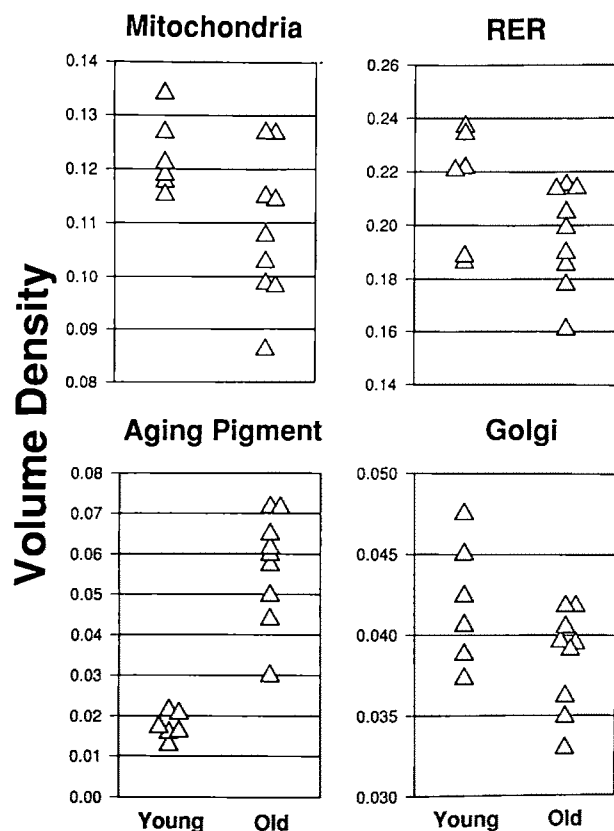


Fig 2. Frequency plots of organelle volume densities. Volume densities of mitochondria, rough endoplasmic reticulum (RER), Golgi apparatus, and aging pigment from old animal group are significantly different from those of young animal group.

plotted according to individual animal age, and a regression analysis was performed (Fig 3). Results indicate that there is a significant increase of aging pigment between 24 and 30 months ($r^2 = .7079$; $p = .0045$; the same type of analysis could not be performed between young and 24-month age groups, since no data were taken between 5 and 24 months).

Statistically significant decreases were present in the volume densities of mitochondria (0.1223 ± 0.0029 young; 0.1084 ± 0.0045 old; $p = .0198$), RER (0.2147 ± 0.0091 young; 0.1955 ± 0.0062 old; $p = .047$), and Golgi apparatus (0.0419 ± 0.0016 young; 0.0385 ± 0.0010 old; $p = .039$). These data are presented in Fig 2. No difference was found for mean diameter. In addition, a K-means cluster analysis was performed (P-KM, BMDP Statistical Software, Los Angeles, Calif) on the young animal data set to determine if the ganglion cells could be categorized on the basis of these variables. The results showed no clear grouping into different cell types.

DISCUSSION

Normally, cells can regulate their metabolism such that the rate of biosynthesis is matched by that of biodegradation and can be varied to meet different

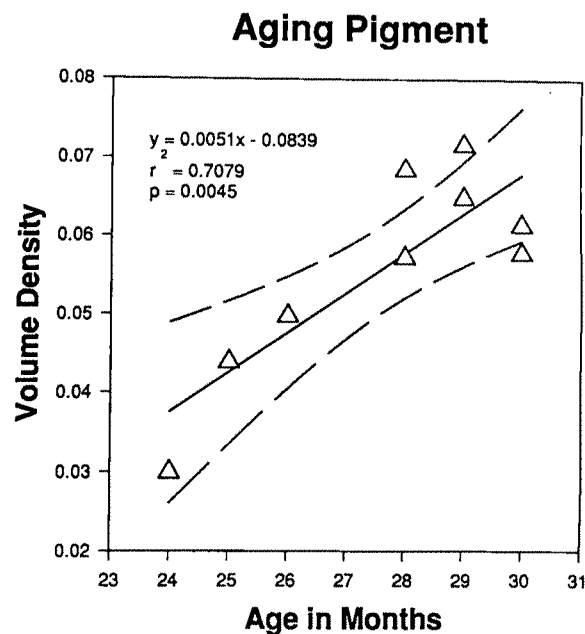


Fig 3. Regression analysis of aging pigment volume density shows significant increase from 24 months to 30 months of age. Rate of accumulation appears to be accelerating in these old animals.

challenges. Aging can be considered as a phenomenon that causes a variety of changes at each level of biologic organization, resulting in a state in which some cells can no longer meet normal metabolic challenges. One proposed aging mechanism that has been rapidly gaining support is the redox mechanism. This combines the theories of free radical and mitochondrial aging. In highly differentiated tissues, utilizing high levels of oxygen, there is release of oxygen free radicals that exceeds the cells' normal protective mechanisms. Free radicals are highly reactive molecules produced along the mitochondrial respiratory chain that can cause protein oxidation and mitochondrial DNA (mtDNA) damage (for review, see Ozawa¹⁹). The mtDNA (located near the inner mitochondrial membrane) is not protected as is nuclear DNA, and mutations are poorly repaired. These mutations may prevent mitochondrial replication and/or expression and eventually cause organelle loss.

Data from the present study, showing a significant decrease in mitochondrial volume density, would suggest that this process is occurring in the rat vestibular ganglion cells. Also, mitochondrial genome products are essential for electron transport and oxidative phosphorylation. It is likely that at least some of the remaining mitochondria are damaged and that adenosine triphosphate (ATP) production is impaired. Therefore, our data are likely to be an underestimation of the total mitochondrial dysfunction. Other data from this study (statistically significant decreases in the RER and Golgi apparatus volume densities)

support this conclusion and indicate an age-related decreased capacity for cellular homeostasis and functional impairment.

Most observations of age-related changes have been interpreted as a sequential degeneration such that ganglion cell loss follows hair cell and nerve fiber degeneration. This may be the case in some of the genetic models of auditory presbycusis such as C57BL/6 mice. However, this is a genetic model and may not truly represent "normal aging." For example, several studies have reported that this strain is more susceptible to noise damage²⁰⁻²² when compared to normal CBA/Ca mice and that this susceptibility increases with increasing age. It is likely that there are other differences in the aging process of these animals. Data from a previous study¹³ as well as the present study suggest that while hair cells may be the first cell type lost in this degenerative sequence, ganglion cell functional impairment at least occurs simultaneously with, and probably precedes, hair cell loss. Nearly all of the aged ganglion cells examined in this study showed a decreased capacity for normal function (ie, protein and ATP synthesis) and a decreased ability to maintain normal peripheral and central connections.

The largest age-related change seen in this study was the accumulation of aging pigment. It accumulates progressively during aging within secondary lysosomes of postmitotic cells in humans and animals²³⁻²⁵ and has frequently been considered a useful aging index. However, there is a great heterogeneity in the structure, composition, and rate of accumulation of aging pigment in different cell types. Within the vestibular system it has been found in the ganglion cells, Schwann cells, hair cells, and supporting cells.^{3,7,11,12,26,27} Hubel and Park¹² described variations in the amount and morphology of aging pigment in the vestibular neuroepithelial cells of mice, as well as specific patterns of accumulation. The melanin-like form was specific for type I hair cells. As pointed out in that study,¹² these differences could be related to differing metabolic pathways or neurotransmitter phenotypes. Data from the study presented here show clear differences in the appearance of the aging pigment within the vestibular ganglion cells. Some cells contained the lipofuscin-like pigment, while others from the same animal contained the melanin-like pigment. On the basis of the data of Hubel and Park,¹² it seems possible that ganglion cells containing the melanin-like pigment innervate type I hair cells, while those containing the lipofuscin-like pigment supply type II hair cells. However, it will take further study to confirm this speculation.

The functional significance of the accumulation of aging pigment is controversial. Some authors believe

that it is detrimental. In fact, one theory of aging, the waste product theory, is partially based on the accumulation of aging pigment.²⁸ Brunk and Collins²⁹ and Brunk and Cadenas³⁰ have postulated that aging pigment may initially provide a secondary antioxidant defense, but that when the total cellular load exceeds a certain threshold, it interferes with essential cellular functions and leads to cell death. Brunk et al³¹ have coupled the free radical theory to the waste product theory. Here, hydrogen peroxide generated by mitochondria interacts with lysosomal ferrous iron to generate hydroxyl free radicals that then induce lipid peroxidation and eventually lipofuscin formation. However, contrary to these theories, the climbing fiber afferents to Purkinje cells that arise in the inferior olive have constant activity levels, even though they have been reported to accumulate more aging pigment in more cells than any other brain structure.^{32,33} The role that aging pigment plays in the vestibular ganglion cells is unknown, and its accumulation accelerates after 24 months of age in the rat. This acceleration may be coupled to the breakdown of cellular mechanisms, leading to mitochondrial dropout and loss of homeostasis capabilities.

In elderly humans, Paige³⁴ has shown that the ability to control eye movements is diminished (particularly at normal head velocities), that the adaptive plastic changes occurring to restore the vestibulo-ocular reflex to normal after a challenge are less effective, and that there is a more pronounced progression of vestibulo-ocular reflex phase change than can be accounted for by cell loss alone. If our data can be extrapolated to the human, then ganglion cell functional impairment preceding cellular loss would help explain the findings of Paige.³⁴ However, this may be too simplistic an interpretation, since other, more central changes may also be contributing to the degradation of these processes.

Pharmacologic treatment with appropriate antioxidants in animals has shown significant decreases of mitochondrial lipid peroxides.^{35,36} Treatment with a radical spin trap has been shown to reverse temporal and spatial memory deficits in gerbils.³⁷ Diet can also influence the progression and severity of age-related degeneration. Park et al³⁸ showed that dietary restriction can significantly decrease the number of spiral ganglion cells lost during aging in mice. While the mechanism(s) responsible for these changes are not clear, these findings do show that pharmacologic therapy coupled with a dietary regimen may be a feasible approach in overcoming some of the manifestations of old age. However, further work must be done to elucidate the mechanisms responsible for these changes before a rational strategy for clinical therapy can be designed.

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EFFICACY OF SINUS IRRIGATION VERSUS SINUS IRRIGATION FOLLOWED BY FUNCTIONAL ENDOSCOPIC SINUS SURGERY

BERT HARTOG, PhD

NIEUWEGEIN, THE NETHERLANDS

LUDO C. PRINS, MD

UTRECHT, THE NETHERLANDS

PETER-PAUL G. VAN BENTHEM, MD, PhD

APELDOORN, THE NETHERLANDS

GERT-JAN HORDIJK, MD, PhD

UTRECHT, THE NETHERLANDS

Sinus irrigation is the traditional treatment for chronic maxillary sinusitis. Functional endoscopic sinus surgery (FESS) restores aeration and allows secretions to be removed from an infected sinus. This study compares the efficacy of sinus irrigation with that of sinus irrigation followed by FESS in 89 patients. We measured the effects by way of sinus radiographs, nasendoscopic findings, and patient complaints. When we analyzed the data in terms of intent to treat, we found significantly favorable results for sinus irrigation followed by FESS at the end point, though only for loss of smell and purulent rhinitis. Treatment consisting of sinus irrigation alone prevented surgery in 58% of all patients for 1 year. Both treatment methods were combined with a 10-day course of loracarbef, which might have contributed to the outcome. We conclude that a good option for treatment of chronic maxillary sinusitis seems to be sinus irrigation in combination with a broad-spectrum antibiotic followed by FESS.

KEY WORDS — chronic maxillary sinusitis, functional endoscopic sinus surgery, loracarbef, sinus irrigation, upper respiratory tract infection.

INTRODUCTION

The traditional treatment for chronic maxillary sinusitis is irrigation and removal of inflammatory products.¹ Over the last decade, functional endoscopic sinus surgery (FESS) — as described by Kennedy et al,^{2,3} Stammberger,^{4,5} and Schaefer et al⁶ — has gained popularity, both in Europe and the United States, as a treatment for chronic maxillary sinusitis. This surgical approach is aimed at restoring the so-called “sinus cycle.”^{7,8} In a normal, healthy sinus, the secretions are constantly removed from the sinus by cilia on the mucosal epithelia. Mucosal swelling may occur in the ostiomeatal region when the mucosa becomes irritated after an allergic reaction or in response to air pollution, for instance, or when a viral infection or rhinitis sets off an inflammatory reaction. In any event, the swelling can block the maxillary ostium. Because of anatomic variations, especially in the size of the natural ostium, such blockage will occur more often in some patients than in others. It leads to pooling of secretions, ciliary dysfunction, a decrease in the oxygen content of the sinus, and a simultaneous increase in its carbon dioxide content. These conditions allow bacteria to flourish and set the stage for an inflammatory process. This vicious circle can be broken by restoring aeration and drainage of the sinus. This is considered to be the most important therapeutic method. Aeration and drainage can both be surgically restored by widening the ostium, eg, by FESS.^{2,3} This technique has been effective in controlled

experiments with rabbits,⁹ while patency rates of up to 94% have been reported in humans.¹⁰⁻¹² Of interest, before the introduction of FESS, many other approaches were being used to treat maxillary sinus disease, including sinus irrigation.^{13,14} Yet sinus irrigation, one of the most fundamental of the “old” techniques, has never been compared with sinus irrigation followed by FESS.

Chronic maxillary sinusitis is a polymicrobial bacterial infection.^{15,16} Therefore, it seems beneficial to combine surgical intervention with a course of antibiotics. If the types of bacteria causing sinusitis are unknown, it is best to use broad-spectrum antibiotics. Loracarbef was chosen for this study, since it is a broad-spectrum antibiotic with proven efficacy on bacteria isolated from patients with upper respiratory tract infections.^{17,18}

In this study, we compare the efficacy of sinus irrigation and sinus irrigation followed by FESS in adult patients with chronic maxillary sinusitis in a prospective, randomized setting.

MATERIALS AND METHODS

This prospective, randomized clinical trial included 89 adult outpatients of 15 otolaryngologists from the otorhinolaryngology departments of 13 hospitals in the Netherlands. The trial lasted 3 years, from March 1992 to April 1995. All patients had diagnoses of chronic maxillary sinusitis, but were otherwise healthy. Patients

From the Department of Otorhinolaryngology, Lucas Hospital, Apeldoorn (van Benthem), and the Department of Otorhinolaryngology, Oudenrijn Ziekenhuis (Prins), and the Department of Otorhinolaryngology, University Hospital Utrecht (Hordijk), Utrecht, the Netherlands. This study was supported by a grant from Eli Lilly Nederland BV.

CORRESPONDENCE — Gert-Jan Hordijk, MD, PhD, Dept of Otorhinolaryngology, University Hospital Utrecht, Heidelberglaan 100, 3584 CX Utrecht, the Netherlands.

TABLE 1. PATIENT DEMOGRAPHICS AT BASELINE

	Sinus Irrigation	Sinus Irrigation and FESS
Sex		
Male	22	22
Female	23	22
Age (y)		
Average	38	35
SD	13.5	13
Weight (kg)		
Average	71	75
SD	10.4	16.3
Antibiotic prescribed before study		
No	11	13
Yes	34	31
Time before study (mo)	1-24	1-18
Patients with known allergies		
No	32	34
Yes	13	10
Duration of complaints (mo)		
Average	31	38
SD	43.2	63.6

FESS — functional endoscopic sinus surgery.

with frontal, ethmoidal, or sphenoidal sinus disease, patients with nasal or ethmoidal polyps, patients with other chronic diseases or immunodeficiencies, and patients who had previously had surgical treatment for their sinusitis (eg, Caldwell-Luc procedure or ethmoidectomy) were excluded from this study. Furthermore, patients with sinusitis that might have been caused by a septal deviation were also excluded. None of the participants were allowed to take concomitant medication with anti-inflammatory effects. The symptoms exhibited at the start of the study included purulent rhinitis, nasal obstruction, and headache; at least two of these three symptoms were obligatory for entry into the study. Participants also reported a loss of their sense of smell, the onset of snoring, and a dry mouth on awakening. The clinical diagnosis of maxillary sinusitis had to be confirmed on sinus radiographs. For this technique, the ailment was defined by the presence of at least one of three features: complete opacity or fluid level or mucosal swelling reaching at least halfway to the mediolateral line of the maxillary sinus. To qualify for entry, each patient had to have been ailing for at least 3 months. In addition, all patients had to be off antibiotics for at least 4 weeks prior to inclusion. After informed consent was obtained, each patient was randomly assigned to one of two treatment groups: sinus irrigation (group A) or sinus irrigation followed by FESS (group B). The patient characteristics at baseline are shown in Table 1.

Study Procedures. At every visit, the investigators

TABLE 2. RESULTS OF SINUS IRRIGATION AND SINUS IRRIGATION FOLLOWED BY FESS ACCORDING TO DEFINITIONS

	Sinus Irrigation	Sinus Irrigation and FESS	
Total No. of patients	45	44	
Nonevaluable	9	3	
Early dropout due to treatment failure	6	1	(p = .110)*
Other reason	3†	2§	
Evaluable	36	41	
Cure	4	6	
Late cure	4	7	
Total cure	8 (22%)	13 (32%)	(p = .444)*
Failure	28	28	
Relapse	2	1	
Recurrence at week 52	0	4	
Lost to follow-up at week 52	4	6	

Evaluable — adhered to inclusion and exclusion criteria; completed 10 days of antibiotic therapy; completed visit at week 12. Cure — completely recovered 6 weeks after treatment. Elimination of clinical signs and symptoms of chronic sinusitis with no recurrence within 12 weeks of therapy. No extra medical help is given (pain medication was allowed during first 2 weeks). Late cure — improvement 6 weeks after treatment. Elimination of clinical signs and symptoms of chronic sinusitis and no recurrence within 12 weeks of therapy. No extra medical help is given (pain medication was allowed during first 2 weeks). Relapse — completely recovered 6 weeks after treatment. Worsening of clinical signs and symptoms of chronic sinusitis within 6 weeks after complete recovery. Medical intervention is necessary. Failure — partial or no improvement 6 weeks after treatment. Extra medical help (including antibiotic therapy) is given within 12 weeks of therapy. Recurrence — after complete recovery within 12 weeks, new signs and symptoms of sinusitis are diagnosed.

*Fisher's exact test.

†Two patients lost to follow-up after week 6; 1 patient missed visit at week 12.

§Two patients lost to follow-up after week 6.

recorded the patients' complaints (purulent rhinitis, snoring, obstruction of the nose, headache, dry mouth on waking, and loss of smell). During the visit, nasendoscopy was performed and the nose was cleaned by removing crusts and secretion. At the start of the study, a sample of sinus fluid was obtained during antroscopy from all patients for aerobic and anaerobic bacterial culture. Each patient was seen for follow-up after 1, 2, 6, 12, and 52 weeks. At each visit, nasendoscopy was performed and the presence of mucosal swelling and nasal secretion was semiquantitatively assessed on a 4-point scale (1 = none, 2 = minimal, 3 = moderate, and 4 = severe). The investigators also recorded the presence of purulency under the middle turbinate. The list of patient complaints was reviewed at each visit, and each complaint was checked off as present or absent. Patients assigned to group A could have a second sinus irrigation after 1 week, if deemed necessary clinically. For patients assigned to group B, FESS was performed within 3 days after enrollment. After 12 weeks, a second sinus

TABLE 3. NASENOSCOPIC FINDINGS OF ALL PATIENTS AT BASELINE AND AFTER 2, 6, 12, AND 52 WEEKS OF FOLLOW-UP

	Sinus Irrigation			Sinus Irrigation and FESS			<i>p</i> *
	No. of Pts	Mean	SEM	No. of Pts	Mean	SEM	
Mucosal swelling							
Baseline	45	2.82	0.10	43	2.86	0.09	.306
Week 2	42	1.80	0.14	41	1.84	0.14	.453
Week 6	41	1.92	0.17	41	1.59	0.14	.134
Week 12	35	1.76	0.16	40	1.61	0.13	.194
Week 52	30	1.72	0.20	34	1.39	0.15	.092
LOCF end point	45	1.91	0.16	43	1.43	0.13	.097
Secretion							
Baseline	45	3.02	0.13	43	2.91	0.12	.322
Week 2	42	1.66	0.15	41	1.81	0.16	.352
Week 6	41	1.85	0.17	41	1.81	0.19	.279
Week 12	35	1.71	0.19	40	1.56	0.18	.477
Week 52	30	1.75	0.21	34	1.47	0.16	.166
LOCF end point	45	1.93	0.17	43	1.51	0.14	.132
Purulence under middle turbinate							
Baseline	45	82%	6%	43	81%	6%	.452
Week 2	42	13%	7%	41	8%	7%	.395
Week 6	41	19%	8%	41	10%	8%	.380
Week 12	35	11%	8%	40	18%	9%	.074
Week 52	30	29%	10%	34	10%	8%	.120
LOCF end point	45	26%	8%	43	7%	7%	.085

Mean and SEM for mucosal swelling and secretion are average scores (1 = none, 2 = minimal, 3 = moderate, 4 = severe), and mean and SEM for purulence are percentages of patients.

Week 2 — mean at 2 weeks (etc), LOCF — last observation carried forward.

*Two-sample F test of sinus irrigation versus sinus irrigation and FESS.

radiograph was made. One year after inclusion in the study, the patients were asked to return for a long-term follow-up visit. Antibiotic therapy (loracarbef, 400 mg twice daily for 10 days) was started for each patient on the day of enrollment into the study. Patients were considered to be evaluable when they met the inclusion and exclusion criteria, after completion of the 10-day course of antibiotics, and after a follow-up of at least 12 weeks. Evaluable patients were classified according to the definitions specified in the note to Table 2.

Functional Endoscopic Surgical Procedure. After local anesthesia of the nasal mucosa with cocaine flakes (200 mg) in combination with adrenaline drops (1:1,000), the lateral nasal wall was injected anterior to the uncinate process with a solution of lidocaine hydrochloride 1% and epinephrine 1:100,000. With a small curved knife, an incision was made just anterior to the uncinate process, cutting downward from above. With a biting forceps, the uncinate process was torn out bluntly. The ostium of the maxillary sinus was localized. With a biting forceps, the ostium of the maxillary sinus was enlarged posteriorly. This maneuver was necessary to open and remove the ethmoid bulla. With a back-biting forceps, the ostium of the maxillary sinus was also enlarged in an anterior direction. The whole procedure

was performed under the control of a rigid nasal endoscope.

Statistical Methods. The results of this study were statistically analyzed for significant differences between the two treatment groups. Fisher's exact test was used to analyze the differences between outcomes according to the set definitions for evaluability and to assess the results of the radiographs. The patient complaints and the nasendoscopic findings were analyzed with the two-sample F test. This test is used for comparing two-population variances. Outcomes in both groups were compared at baseline and after 2, 6, 12, and 52 weeks of follow-up. Also, an intent to treat (last observation carried forward; LOCF) analysis was done for both nasendoscopic findings and patient complaints with the two-sample F test.

This study was performed and monitored in accordance with the European Guidelines for Good Clinical Practice.¹⁹ The study protocol was approved by the local medical ethical committees (institutional review boards) at all participating hospitals. Informed consent was obtained from all patients before they were enrolled in the study.

RESULTS

We started this trial with a total of 89 patients: 45 in

TABLE 4. PATIENT COMPLAINTS REPORTED AT BASELINE AND AFTER 2, 6, 12, AND 52 WEEKS OF FOLLOW-UP

	Sinus Irrigation			Sinus Irrigation and FESS			<i>p</i> *
	No. of Pts	Mean	SEM	No. of Pts	Mean	SEM	
Purulent rhinitis							
Baseline	45	91%	4%	43	86%	5%	.099
Week 2	42	20%	7%	41	37%	9%	.114
Week 6	42	34%	9%	41	23%	8%	.115
Week 12	37	15%	7%	40	26%	8%	.214
Week 52	31	39%	12%	35	15%	8%	.015
LOCF end point	45	40%	9%	43	16%	7%	.027
Snoring							
Baseline	45	33%	7%	43	47%	8%	.354
Week 2	42	9%	7%	41	18%	8%	.138
Week 6	42	16%	8%	41	13%	9%	.334
Week 12	37	14%	9%	40	12%	9%	.251
Week 52	31	14%	9%	35	10%	9%	.226
LOCF end point	45	17%	6%	43	12%	8%	.074
Obstruction of nose							
Baseline	45	89%	5%	43	91%	5%	.306
Week 2	42	18%	7%	41	28%	8%	.341
Week 6	42	41%	9%	41	23%	8%	.205
Week 12	37	32%	9%	40	26%	8%	.199
Week 52	31	21%	9%	35	20%	8%	.417
LOCF end point	45	33%	8%	43	19%	7%	.116
Headache							
Baseline	45	89%	5%	43	81%	6%	.081
Week 2	42	37%	8%	41	30%	9%	.147
Week 6	42	29%	8%	41	25%	9%	.260
Week 12	37	11%	7%	40	26%	9%	.046
Week 52	31	24%	10%	35	7%	8%	.111
LOCF end point	45	25%	8%	43	9%	7%	.160
Dry mouth on waking							
Baseline	45	53%	8%	43	65%	7%	.385
Week 2	42	15%	9%	41	33%	9%	.435
Week 6	42	27%	8%	41	23%	10%	.094
Week 12	37	39%	9%	40	20%	10%	.145
Week 52	31	5%	10%	35	25%	10%	.376
LOCF end point	45	15%	9%	43	23%	10%	.448
Loss of smell							
Baseline	45	49%	8%	43	51%	8%	.498
Week 2	42	6%	8%	41	12%	9%	.159
Week 6	42	28%	10%	41	5%	8%	.062
Week 12	37	11%	10%	40	6%	8%	.157
Week 52	31	10%	11%	35	11%	8%	.115
LOCF end point	45	18%	10%	43	11%	8%	.026

Data are percentages of patients.

Week 2 — mean at 2 weeks (etc), LOCF — last observation carried forward.

*Two-sample F test of sinus irrigation versus sinus irrigation and FESS.

group A (sinus irrigation only) and 44 in group B (sinus irrigation followed by FESS). The two treatment groups were comparable in size, sex, weight, antibiotic use before study start, known allergies, and average dura-

tion of complaints (Table 1). Seventy-seven (77) patients turned out to be evaluable for efficacy analysis according to the criteria mentioned in the note to Table 2. The efficacy results of these patients are depicted in

Fig 1. Nasendoscopy findings of mucosal swelling and purulency under middle turbinate. Results of all patients at baseline and after 2, 6, 12, and 52 weeks of follow-up.

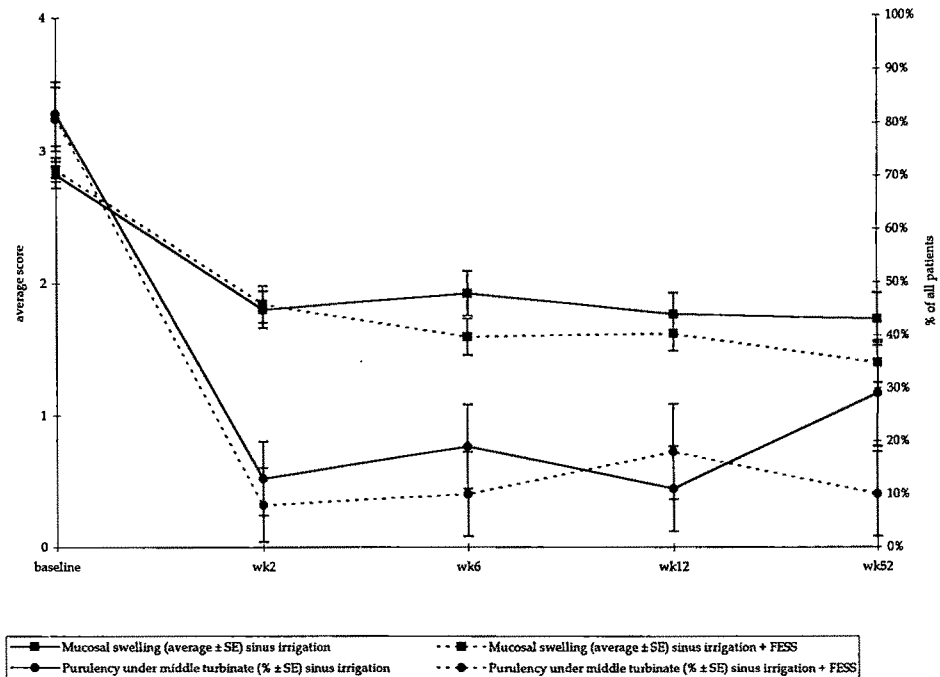


Table 2.

A total of 14 patients were lost to follow-up during the study: 4 at week 6, and another 10 at week 52. Table 3 shows the objective results, as obtained by nasendoscopy, on the efficacy of both treatment methods for all patients. The subjective results, as shown by the complaints reported by the patients, are depicted in Table 4. Tables 3 and 4 show baseline values and scores after 2, 6, 12, and 52 weeks. The objective and subjective results could not always be obtained from all patients at each visit, for several reasons (eg, patient cooperation was needed to perform nasendoscopy). This explains

the difference in patient numbers in the overview of visits in Tables 3 and 4. The results of the intent to treat analysis (LOCF) are shown in these Tables. One patient of group B was left out of the LOCF analysis, because no post-baseline data could be obtained. At diverse points in time, no statistically significant differences ($p < .05$) were found between the two treatment groups. Only the differences in the patient complaints of purulent rhinitis ($p = .027$) and loss of smell ($p = .026$) were statistically significant in the intent to treat analysis.

Figure 1 shows the severity of mucosal swelling and the presence of purulency under the middle turbinate as

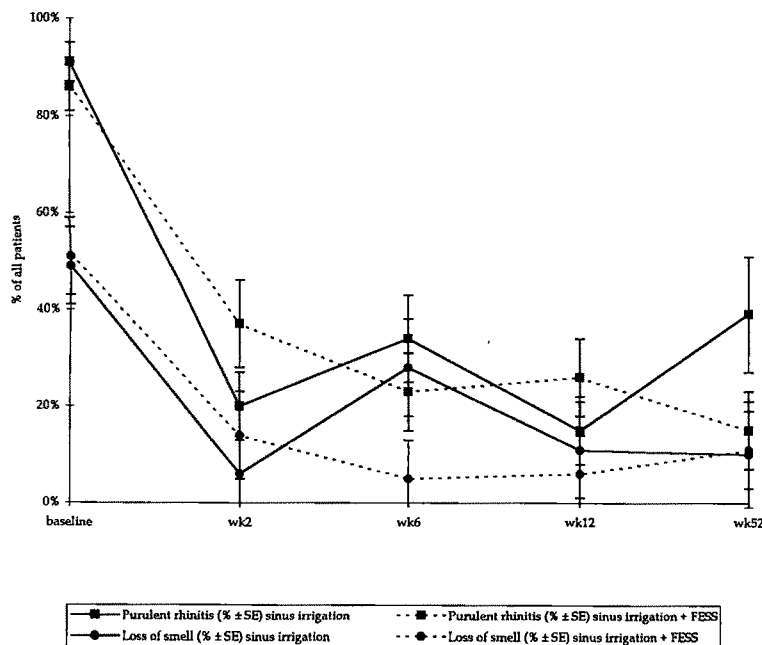


Fig 2. Patient complaints (purulent rhinitis and loss of smell) reported at baseline and after 2, 6, 12, and 52 weeks of follow-up.

TABLE 5. SINUS RADIOGRAPH EVALUATIONS OF ALL PATIENTS AT BASELINE AND AFTER 12 WEEKS OF FOLLOW-UP

	Sinus Irrigation		Sinus Irrigation and FESS		<i>p</i> ^c
	Baseline	Week 12 ^a	Baseline	Week 12 ^b	
Complete opacity	56% (25/45)	0%	50% (22/44)	11% (4/37)	.115
Fluid level	31% (14/45)	0%	27% (12/44)	5% (2/37)	.493
Mucosal swelling ^d	62% (28/45)	37% (13/35)	68% (30/44)	41% (15/37)	.812
Normal sinus radiograph	0%	63% (22/35)	0%	51% (19/37)	.351

Data are percentages of patients. Numbers and totals are in parentheses.

^aSinus radiographs were obtained after 12 weeks for 35 of 36 evaluable patients.

^bSinus radiographs were obtained after 12 weeks for 37 of 41 evaluable patients.

^cFisher's exact test on week 12 results, sinus irrigation versus sinus irrigation and FESS.

^dDefined as swelling of at least half of mediolateral diameter of maxillary sinus.

assessed by nasendoscopic examinations performed at baseline and after 2, 6, 12, and 52 weeks of follow-up. Statistical analysis (two-sample F test) of the results at the different points in time, and the intent to treat analysis (LOCF) did not show any significant difference between the two treatment methods at the $p < .05$ level.

Figure 2 shows the results of the reported patient complaints, purulent rhinitis and loss of smell, at baseline and after 2, 6, 12, and 52 weeks of follow-up. Both improved after treatment. Statistical analysis of the improvement from baseline revealed significant differences between the two treatment groups for purulent rhinitis only, at week 52. The intent to treat analysis (LOCF) showed a statistically significant difference between the treatment groups for both purulent rhinitis and loss of smell. The presence of purulent rhinitis decreased from 91% to 40% after sinus irrigation and from 86% to 16% after sinus irrigation followed by FESS ($p = .027$). The incidence of loss of smell decreased from 49% to 18% after sinus irrigation and from 51% to 11% after sinus irrigation followed by FESS ($p = .026$). The results of sinus radiographs taken at baseline and after 12 weeks follow-up are presented in Table 5.

No major surgical complications were reported. One patient did complain of numbness following antroscopy. For this patient, antroscopy was performed through the canine fossa. The numbness disappeared after a few weeks. In 1 case, antroscopy could not be performed due to the thickness of the lateral nasal wall. A total of 20 patients reported adverse events during the course of antibiotic treatment (6 rash or allergic reactions, 9 gastrointestinal complaints, 2 headaches, 1 dizziness, 1 tiredness, and 1 loss of smell). All events were mild and transient. The complaints disappeared spontaneously after the end of the treatment course. No medical interventions were deemed necessary for treatment of any of these events. All patients completed the 10-day course of loracarbef.

In 13 cases in group A, a second sinus irrigation was

performed after 1 week. Due to lack of efficacy, surgery was necessary in 15 patients. Of these patients, 13 were from group A. Nine of them underwent FESS: 1 after 2 weeks, 2 after 6 weeks, 2 after 3 months, and 4 after 1 year. Two of the 13 had antrostomies under the inferior turbinate: 1 after 6 weeks, and the other after 3 months. Two had unspecified surgical interventions: 1 after 6 weeks, and the other after 3 months. The remaining 2 patients of the 15 were from group B. Both of them repeated FESS after 3 months.

The results of the bacteriologic cultures showed bacterial strains to be present in 81% of all samples taken at the start of the study. An average of 1.9 different strains were cultured per sample. A total of 29% of all samples contained anaerobic bacteria: 5% pure anaerobic, and 24% mixed aerobic-anaerobic. Pure aerobic cultures were found in 52% of all samples.¹⁸ The two treatment groups did not differ with regard to the results of the bacteriologic cultures.

DISCUSSION

Functional endoscopic sinus surgery has increased in popularity ever since it was introduced in the late 1970s.²⁰ The comprehensive studies and fundamental considerations published by Stammberger et al^{4,5,21} and Kennedy et al^{2,3} contributed to its popularity. It is based on the pathophysiology of sinusitis, in which both aeration and drainage through the ostium are impaired, or the ostium is completely blocked. The mucosal clearance pattern in the sinus is highly specific and requires effectively functioning cilia.^{7,9} Antrostomy at different locations in the lateral wall of the maxillary sinus has been found to disturb this clearance pattern. Experiments in rabbits showed that widening of the natural ostium resulted in a restored, although typically imperfect, mucociliary clearance through the ostium.²² Some risks associated with FESS have been reported. According to Stammberger and Posawetz²¹ and Maniglia,²³ this technique needs to be kept in the hands of well-trained physicians; it should not be used on all patients in just any clinical setting. Many sets of results

of this technique have been published, with patency rates between 80% and 98%.^{10-12,24}

According to the definitions of cure and late cure, as given in the note to Table 2, a total cure rate of 32% was found in patients treated by sinus irrigation followed by FESS, compared to 22% in the other group. The number of patients who could not be evaluated due to treatment failure was much lower in the group on which FESS was performed (1 versus 6). These findings seem to indicate a better efficacy for sinus irrigation followed by FESS, although the difference is not statistically significant. The total cure rate of 22% for patients treated by only sinus irrigation cannot be explained by the possibility of spontaneous cure in chronic sinusitis as described by Mann and Jonas.²⁵ The population they described consisted of 15 patients with "a long-lasting sinusitis," headache, secretion, and abnormal radiographic findings. On the basis of this description, it is difficult to compare this population with the group of patients we studied. All patients in our study had been suffering from sinusitis for a long time (on average, more than 30 months), and most of them had been treated by antibiotics before. A spontaneous cure would therefore be unlikely. The efficacy of sinus irrigation might be explained by the regenerative capacity of the sinus mucosa, in combination with the reported finding that bacteria are located in sinus secretions and not in the mucosa.²⁶ Sinus irrigation to clear the sinus of secretions would thus remove the bacteria from the sinus. The antibiotic that was given to all patients in our study could have killed any remaining bacteria. This might have been sufficient, in a number of patients, to allow the sinus mucosa to restore itself and thus relieve the patients of the signs and symptoms of sinusitis.

As far as we know, no comparison between FESS and sinus irrigation has ever been reported. In our study, all patients showed good overall improvement after 12 weeks of follow-up. Improvements in nasal obstruction, headache, rhinitis, loss of smell, dry mouth on waking, and snoring were reported by a majority of patients in both treatment groups. Nasendoscopic findings improved in both treatment groups during the 1-year follow-up. It is unclear why the intent to treat analysis of two patient complaints — purulent rhinitis and loss of smell — shows statistically significant differences between the two treatment groups. These findings are not supported by the other measurements, eg, obstruction of the nose and mucosal swelling. The sinus radiographs confirmed the observed and reported improvement of signs and symptoms of sinusitis. After 12 weeks of follow-up in the group of patients treated by only sinus irrigation, opacity and fluid level were no longer visible on the sinus radiographs. The incidence of opacity and fluid level on sinus radiographs in the

was much lower after 12 weeks of follow-up than at baseline. The presence of mucosal swelling on the sinus radiographs decreased in both treatment groups, although the swelling did not disappear after 12 weeks of follow-up. Seven of the 8 patients who were treated by sinus irrigation only and who fulfilled the criteria for cure or late cure had normal radiographs after 12 weeks of follow-up; the remaining patient still had mucosal swelling. There were 13 patients who were treated by sinus irrigation followed by FESS and fulfilled the criteria for cure or late cure. Of these, 8 had normal radiographs after 12 weeks of follow-up, 4 still had mucosal swelling, and 1 showed complete opacity. It has been demonstrated in previous studies^{27,28} that the correlation between radiographic findings and the presence of clinical signs and symptoms is less than 100%. Our results are in line with this finding.

In this study, a total of 20 patients reported adverse events during the course of the antibiotic. A meta-analysis of clinical trials comparing the safety and efficacy of loracarbef with those of several other antibiotics showed that one or more adverse events were reported by 26% of patients treated with loracarbef and by 29% of patients treated with comparator antibiotics.¹⁷ The number of adverse events in this study (20/89 or 22%) is in line with the numbers reported in previous studies.

The patients treated by sinus irrigation only (group A) needed surgical interventions in 13 of 45 cases, compared to 2 of 44 in the other group. The other 32 patients in group A required no further treatment for their sinusitis (26) or were lost to follow-up (6). Thus, in 26 of 45 cases (58%), sinus irrigation proved to prevent the need for sinus surgery within 1 year. This figure is much higher than the 36% reported by Bertrand and Eloy²⁹ (their patients treated by repeated sinus irrigation for whom surgery was not necessary). This difference might be due to the combined treatment by sinus irrigation and antibiotics that we used in our study. In the study by Bertrand and Eloy, the patients were treated by repeated daily sinus irrigation for 21 days, but no antibiotics were prescribed. The antibiotic might be essential in destroying any bacteria left after sinus irrigation. This, in turn, would remove the inflammatory stimulus of the presence of bacteria in the sinus.

The long-term efficacy of both treatment methods does not seem to differ much from the short-term (12 weeks of follow-up) results. In the group of patients treated by sinus irrigation and FESS, 4 of the 13 cured patients suffered a recurrence during the 1-year follow-up. After 1 year of follow-up, the 8 patients who were considered cured after treatment by sinus irrigation alone still did not show any signs or symptoms related

onstrate the need for long-term follow-up, even when a total cure has been achieved within 3 months after treatment.

CONCLUSIONS

Sinus irrigation and sinus irrigation followed by FESS both led to overall improvement in the signs and symptoms of chronic sinusitis in this clinical trial. Direct comparison of the results of both treatment methods shows a tendency toward favorable results after sinus irrigation followed by FESS. This tendency can be seen in the intent to treat analysis on all end points, although it is only statistically significant ($p < .05$) for improvement in purulent rhinitis and loss of

smell. However, given the risks associated with FESS and in light of our finding that treatment by sinus irrigation alone prevented surgery in 58% of patients, we conclude that sinus irrigation is still a suitable treatment for patients with sinus disease that is limited to the maxillary sinus. Simultaneous antibiotic therapy seems to be beneficial in both treatment methods. Our recommendation for treating patients with chronic maxillary sinusitis therefore is to take a two-step approach. The first step is to treat patients by performing sinus irrigation and prescribing a broad-spectrum antibiotic. If the results of this treatment are unsatisfactory, the second step would be to perform sinus surgery, specifically, FESS.

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LOCAL AND REGIONAL ANESTHESIA FOR FUNCTIONAL ENDOSCOPIC SINUS SURGERY

WAI CHUNG LEE, FRCS

DUDLEY, ENGLAND

TILAK R. KAPUR, FRCS

STOKE-ON-TRENT, ENGLAND

WALTER N. RAMSDEN, FRCA

STOKE-ON-TRENT, ENGLAND

An effective topical and regional anesthetic technique using 25% cocaine paste combined with intravenous midazolam hydrochloride was used in 554 patients undergoing functional endoscopic sinus surgery. The operative fields were excellent and all procedures were completed with little patient disturbance. Patients recovered rapidly from sedation and were usually fit for discharge on the same day. There were no anesthetic complications. The major surgical complication rate was 0.5%.

KEY WORDS — complications, functional endoscopic sinus surgery, local anesthesia, sedation.

INTRODUCTION

Functional endoscopic sinus surgery (FESS) is performed either under general anesthesia with local use of vasoconstrictors with or without hypotension, or under local anesthesia. In the United Kingdom, it tends to be performed under general anesthesia.¹ This may be due to the preference of the surgeons or their fear of patient anxiety, pain, and bodily movements if a purely local anesthetic approach is used. However, the long-term experience with local anesthesia for FESS in Europe, the United States, and in this center suggests that the problem of intraoperative patient disturbance is overstated. In this report, our experience of a local and regional anesthetic technique for FESS under midazolam hydrochloride sedation is described.

MATERIALS AND METHODS

Each patient received an intramuscular premedication consisting of an opiate (morphine sulfate 10 mg or Papaveretum 15.4 mg, depending on the patient's weight) and droperidol 2.5 mg. A small dose of intravenous (IV) midazolam hydrochloride, usually 2.5 mg, was administered in the anesthetic room just before the local anesthesia.

A small volume of 25% cocaine paste (25% cocaine hydrochloride and 0.01% epinephrine acid tartrate in paraffin paste, produced by the manufacturing unit of the pharmacy at North Staffordshire Royal Infirmary) was placed onto a small piece of moist cotton mounted on a Tumarkin wire. The anterior septum, anterior ends of the middle and inferior turbinates, and lateral nasal wall were painted gently

with the applicator. The wire was left between the nasal bone and the septum to anesthetize the anterior ethmoidal nerve. The mucosa lining the middle portion of the nasal cavity was then painted with a second applicator that was finally inserted into the middle meatus. A third applicator was inserted posteriorly to rest in the region of the sphenopalatine foramen behind the middle turbinate after the lining of the posterior nasal cavity had been painted with more cocaine paste. Three wires were used for each side. A maximum of 500 mg of 25% cocaine paste (containing 125 mg cocaine hydrochloride) was used. The technique was suitable for extensive endoscopic surgery. Septal infiltration with prilocaine hydrochloride with felypressin was added if septoplasty was deemed necessary to gain access to the ethmoidal region. Before surgery, 15 to 20 minutes were given for the local anesthetic to take effect.

Although the nasal mucosa was anesthetized, patient anxiety was minimized by maintenance of intraoperative sedation with further doses of midazolam as necessary. Usually less than 10 mg of midazolam was required for the whole procedure, which could last up to 1½ hours in the most difficult cases. Spontaneous respiration, pulse, blood pressure, oxygen saturation, electrocardiogram, and level of sedation were carefully monitored by an anesthetist (W.N.R.). The level of sedation was such that the patients were able to respond to simple commands, but were otherwise still or nearly still throughout the procedure. Provided verbal contact was maintained, any respiratory depression (which was detected by a fall in oxygen saturation) could be immediately corrected by asking the patient to breathe deeply.

From the Department of Otolaryngology, North Staffordshire Royal Infirmary, Stoke-on-Trent, England.

CORRESPONDENCE — Wai Chung Lee, FRCS, Dept of Otolaryngology, Russells Hall Hospital, Dudley, DY1 2LU, England.

RESULTS

The technique was used in 554 patients over a 5-year period, and of these, 342 had ethmoidectomy (unilateral or bilateral) with or without sphenoidal sinus exploration. The remaining 212 patients underwent other procedures such as uncinctomy, excision of the lateral lamella of a concha bullosa, or excision of Heller cells or diseased anterior ethmoidal cells causing frontal sinus disease. In no case was the operation incomplete or abandoned due to excessive bleeding or lack of patient cooperation. Invariably, the patients had marked amnesia for the operation. Tachycardia (>100 beats per minute) occurred in fewer than 10% of patients and was treated with IV propranolol hydrochloride. No adverse reaction to cocaine occurred. Cardiac arrhythmias had been notable for their total absence.

Perioperative surgical complications were rare. General anesthesia had to be used at the end of an ethmoidectomy in only 1 patient to control orbital hemorrhage due to a torn and retracted anterior ethmoidal artery. A cerebrospinal fluid (CSF) leak occurred in 1 elderly patient that was recognized intraoperatively. The leaky area was sealed with inferior turbinate mucosa and packed. Three patients were noted to have a tiny intranasal orbital fat prolapse intraoperatively that was completely asymptomatic. Two patients developed a self-limiting orbital emphysema following nose-blowing that resolved spontaneously. Nasal packing and blood transfusion were necessary in 1 patient due to significant reactive hemorrhage. No postoperative CSF rhinorrhea or other major complications were encountered, and the major surgical complication rate was 0.5% in this series (orbital fat prolapse and emphysema excluded).

DISCUSSION

A number of local anesthetic agents are applied in FESS, usually in combination with mucosal infiltration of lidocaine hydrochloride and epinephrine. Kennedy² described a technique using topical cocaine and mucosal infiltration of 1% lidocaine with epinephrine 1:100,000. Others described the use of a mixture of tetracaine chloride and epinephrine and cocaine chloride with IV diazepam and fentanyl³ or 2% Pontocaine and epinephrine without IV drug administration⁴ followed by mucosal infiltration of 1% lidocaine with epinephrine 1:200,000. A vasoconstrictor nasal spray combined with topical application of 1% lidocaine with epinephrine 1:100,000 followed by mucosal infiltration of the same local anesthetic has also been described.⁵ In our study, a method using 25% cocaine paste locally with IV sedation but without

mucosal injection was used.

General anesthesia with cocainization and hypotension was used in 172 patients undergoing FESS in this institution over a 2-year period. In 1990, the senior author (T.R.K.) adopted the local and regional anesthetic technique with IV sedation. Previously, under general anesthesia, the operative field was bloody. This was vastly improved with the local and regional technique, so that the need for rod lens cleaning and suction and the operating time were reduced. Furthermore, pain alerted the surgeon to any transgression of sinus boundaries, thereby contributing further to operation safety. The patients accepted the technique well, with minimal disturbance with regard to nausea, vomiting, headache, and bleeding, and most were able to go home on the same day without any nasal packing. The technique was used in adults only (18 to 72 years old).

The higher concentration of water-soluble cocaine and epinephrine salts is necessary in a solid dispersible preparation in order to produce the desirable pharmacologic effects. The maximum safe dose of cocaine remains uncertain, and figures quoted have varied between 100 and 200 mg for adults. Although a maximum of about 500 mg of 25% cocaine paste was used (125 mg cocaine), a significant amount was removed during the withdrawal of the cotton applicators. Some of the paste left on the mucosa was also aspirated at the start of the endoscopic procedure. This maneuver appears to reduce cocaine (and epinephrine) absorption, thereby increasing the safety margin of these drugs. Topical cocaine and epinephrine nasal preparations in use have been shown to reduce the systemic level of cocaine as compared to using cocaine topically alone, though both showed no toxicity,⁶ and this may explain the lack of toxicity of the cocaine paste that was used extensively in this series.

A lack of careful monitoring during sedation and recovery can result in fatality, as shown in patients undergoing gastrointestinal endoscopy.⁷ The complete absence of any serious or fatal cardiovascular or respiratory complications in this series clearly supports perioperative monitoring.

CONCLUSION

In this study a simple, relatively safe, and effective technique of local and regional anesthesia with IV sedation for endoscopic sinus surgery was described. The advantage over general anesthesia has been highlighted. The importance of meticulous local anesthesia and allowance of sufficient time for it to act, as well as the presence of an anesthetist with full monitoring facilities, has been emphasized.

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ENDOSCOPY DAYS IN PEDIATRIC OTOLARYNGOLOGY

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IDIOPATHIC SUBGLOTTIC STENOSIS: DIAGNOSIS AND ENDOSCOPIC LASER TREATMENT

BRUCE BENJAMIN, FRACS

IAN JACOBSON, FRACS

ROBERT ECKSTEIN, FRCPA

SYDNEY, AUSTRALIA

The records of 15 patients with idiopathic subglottic stenosis treated at Royal North Shore Hospital, Sydney, between 1980 and 1994 were reviewed. All were female and had similar characteristic clinical and histopathologic features. Endoscopic laser vaporization was the primary treatment in 12 patients and was successful in maintaining the airway of 8 of these 12; this outcome indicates that the disease can be managed, at least initially, by endoscopic laser treatment in most cases.

KEY WORDS — endoscopic laser treatment, idiopathic subglottic stenosis.

Idiopathic subglottic stenosis (ISS) is a rare, slowly progressive inflammatory process of unknown cause, limited to the subglottic region and first tracheal arch and apparently occurring only in females. There are no indicators of precipitant or concurrent disease, either at the time of presentation or subsequently. The features are stridor, dyspnea, sometimes dysphonia, and occasionally life-threatening airway obstruction. Idiopathic subglottic stenosis has been reported by others¹⁻⁵; the largest series⁴ emphasized the localized subglottic nature of the stenosis, the undetermined cause, and the difficulties with treatment. Favorable results were reported in selected cases with use of single-stage resection of the stenosis and primary reconstruction of the airway.

An association between gastroesophageal reflux and ISS has been proposed based on clinical⁵ and experimental⁶ observations, but reflux is certainly not present in all patients. An autoimmune cause has been suggested for some cases,⁷ but remains unproven. Thus, the cause of the condition is unknown.

We report a consecutive series of 15 female patients with ISS, seen by one clinician (B. B.) over a period of 15 years, and highlight the clinical features and response to laser therapy.

MATERIALS AND METHODS

A retrospective analysis of 15 patients treated for ISS between 1980 and 1994 was performed with regard to age at presentation, nature and duration of symptoms, previous local trauma (including endotracheal intubation), and systemic illness. The radiologic and endoscopic appearances were reviewed.

Histopathologic features of biopsies from all 15 patients were compared, and sections from 10 patients were analyzed for estrogen receptor status. The microbiology of biopsy specimens and the immunologic investigations performed were noted. The effectiveness of therapeutic modalities employed, especially treatment using the carbon dioxide laser, was examined.

Lateral airway x-rays (Fig 1) confirmed subglottic stenosis and provided an appreciation of its extent, but the severity of airway stenosis was more accu-

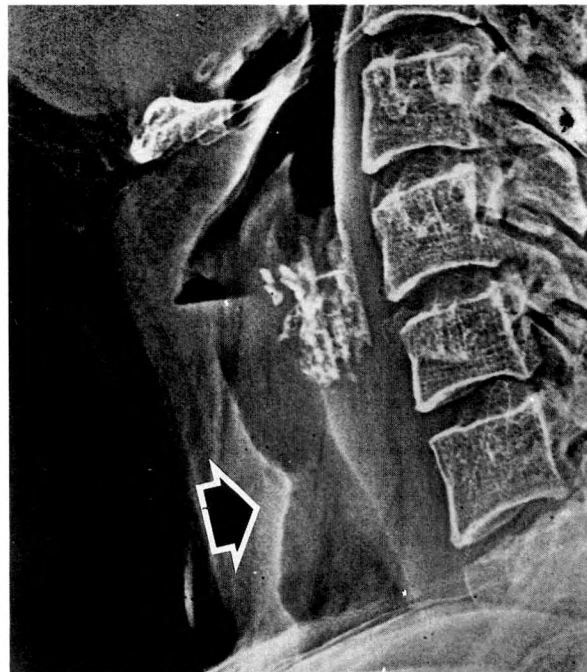


Fig 1. Lateral soft tissue radiograph showing site of stenosis (arrow), more prominent anteriorly in this patient.

From the Departments of Otolaryngology-Head and Neck Surgery (Benjamin, Jacobson) and Anatomical Pathology (Eckstein), Royal North Shore Hospital, St Leonards, Sydney, Australia.

CORRESPONDENCE — Bruce Benjamin, FRACS, 231 Macquarie St, Sydney, NSW 2000, Australia.



Fig 2. Subglottiscope in position. Hyperextension of neck and positioning of laryngoscope accentuates posterior aspect of stenosis preparatory to laser vaporization. Distal end of metal jet ventilation cannula is seen on left, within subglottiscope.

rately measured by calibration in millimeters during endoscopy. A three-stage laryngoscopic procedure,^{8,9} employing a variety of straight-ahead and angled rigid rod lens telescopes under general anesthesia, provided detailed examination of the larynx and trachea.

The carbon dioxide laser was used to treat most patients, with jet ventilation anesthesia and a slim microlaryngoscope positioned as a subglottiscope¹⁰ (Fig 2). To reduce the risk of circumferential cicatricial contraction, a series of planned, staged procedures was performed. No more than one third of the circumference of the stenosis was vaporized at any procedure, and care was taken to avoid exposure of underlying cartilage. In 1 patient with severe obstruction, a temporary tracheotomy was performed before the first laser treatment. Open surgical procedures, either laryngotracheoplasty or insertion of a "permanent" tubular silicone stent, were employed for 2 cases that progressed despite repeated laser treatment. A laryngotracheoplasty using rib cartilage was performed on 1 patient as the primary treatment, on what later proved to be an erroneous assumption that the stenosis was fibrous and due to intubation performed 5 years previously.

RESULTS

All patients were women (see Table). Their ages at presentation ranged from 15 to 66 years (mean 43

years). All had dyspnea and progressive stridor. Five had some voice change. The duration of symptoms prior to presentation varied between 1 week and 9 years (mean 31 months).

None of the patients had sustained cervical injury or been intubated for a prolonged period. Five had been intubated for brief general anesthesia between 4 and 10 years prior to the onset of their symptoms. One patient had had diphtheria as a child and also suffered mild rheumatoid arthritis. None had Wegener's granulomatosis, relapsing polychondritis, or other connective tissue disease. Two patients reported occasional, mild symptoms of gastroesophageal reflux.

The endoscopic appearance was similar in all cases. The supraglottic and glottic larynx were normal. Neither vocal cord nor cricoarytenoid joint mobility was impaired. The stenosis commenced in the immediate subglottic region and extended to the inferior margin of the cricoid cartilage or to the first tracheal arch. Calibration was performed with the Benjamin-Jackson esophageal bougies⁹; the diameter at the narrowest point (except for patient 1, who had had a tracheotomy for complete occlusion) was from 3.5 to 7.0 mm at first endoscopy, all lesions being circumferential, although some were more prominent in one aspect than another (Fig 1). The mucosa was reddened and had a finely granular inflammatory appearance, the underlying tissue was soft to firm, and the surface bled easily on manipulation or biopsy. The trachea distal to the stenosis was normal.

There was a characteristic histopathologic appearance (Fig 3). The most striking finding common to all biopsies was dense fibrous tissue, extending to the epithelial surface. The fibrosis was generally poorly cellular and the collagen very dense, although in some areas greater cellularity resembled fibromatosis. Blood vessels were scanty and tended to be surrounded by a mixed mononuclear inflammatory cell infiltrate. Some of the more superficial biopsies showed edematous tissue with a heavy mixed inflammatory infiltrate and granulation tissue. The overlying epithelium was of squamous, respiratory, or transitional type and, although sometimes hyperplastic, was never dysplastic. No vasculitis, granuloma formation, or foreign body was seen. Congo red stain for amyloid was negative. Acid-fast bacilli and fungi were not demonstrated. An estrogen receptor assay was performed retrospectively in 10 cases by an immunohistochemical technique¹¹ and was negative in all. Routine culture of all biopsies was negative. Cytoplasmic and perinuclear pattern antineutrophil cytoplasmic autoantibodies (cANCA and pANCA) were absent in the 5 patients tested.

FIFTEEN PATIENTS TREATED FOR IDIOPATHIC SUBGLOTTIC STENOSIS

Patient	Age at Presentation	Presenting Symptoms	Duration of Symptoms	Previous Intubation	Initial Size (mm)	Previous Treatment	Treatment	Permanent* Tracheotomy	Time Under Review
1	49	Stridor, dysphonia	3 y	Yes	Complete occlusion	Failed laryngo-tracheoplasty	Laser, laryngofissure, permanent stent	Yes	2 y
2	66	Stridor, dysphonia	4 y	Yes			Laser	No	7 mo
3	21	Stridor, dysphonia	4 y	No	6.5		Laser	No	2.5 y
4	15	Stridor	1 y	No	5		Laser	No	2 y
5	25	Stridor, dysphonia	9 y	No	3.5	Failed laryngo-tracheoplasty twice, radiotherapy		Yes	
6	63	Stridor	"Months"	No	7		Observation	No	1.5 y
7	34	Acute on chronic stridor	7 d	Yes	5		Laser	No	6 mo
8	47	Stridor	1 y	Yes	4		Laser, laryngo-tracheoplasty	Yes	2.5 y
9	37	Stridor, cough	1 y	No	5		Laser	No	8 y
10	43	Stridor	2 y	No			Laser	No	7 y
11	19	Stridor, dysphonia	4 mo	No			Laser	Yes	5 y
12	63	Stridor	3 y	No			Laser, laryngofissure, stent	Yes	7 y
13	46	Stridor	1 mo	Yes			Laser, laryngofissure, stent	Yes	10 y
14	64	Acute on chronic stridor	6 y	No	4		Laser	No	7 y
15	46	Stridor	4 y	No	5	Dilation	Laser	No	6 mo

*Patient cannot be decannulated.

Two patients had been treated prior to referral. The first had had autogenous costal cartilage graft laryngotracheoplasty that failed and she was tracheotomy-dependent. Laser treatment was unsuccessful, and she was ultimately managed by insertion of an endolaryngeal stent to maintain a phonatory airway. The second had undergone two prior laryngotracheoplasties and had been treated with radiotherapy in a vain attempt to halt progression of the disease. She declined further surgery and was managed by tracheotomy alone.

One patient with a mild subglottic stenosis and minor symptoms underwent only diagnostic assessment including biopsy. She has been followed up for 18 months and has remained stable.

One patient presented acutely, required emergency

tracheotomy, and was then treated by laser vaporization on five occasions over a 22-month period before successful decannulation. She has been followed up for 5 years and remains symptom-free.

The remaining 11 patients were treated from the beginning with endoscopic laser surgery. Five were treated effectively with laser alone, requiring between two and eight procedures. Two further patients required temporary tracheotomy during the course of their laser therapy and were subsequently decannulated. One patient remains under treatment and still has a tracheotomy. Laryngotracheoplasty was performed in 1 patient on the erroneous assumption that she had fibrous subglottic stenosis, but this procedure proved to be inadequate; she had several subsequent attempts at laser treatment and now has a permanent

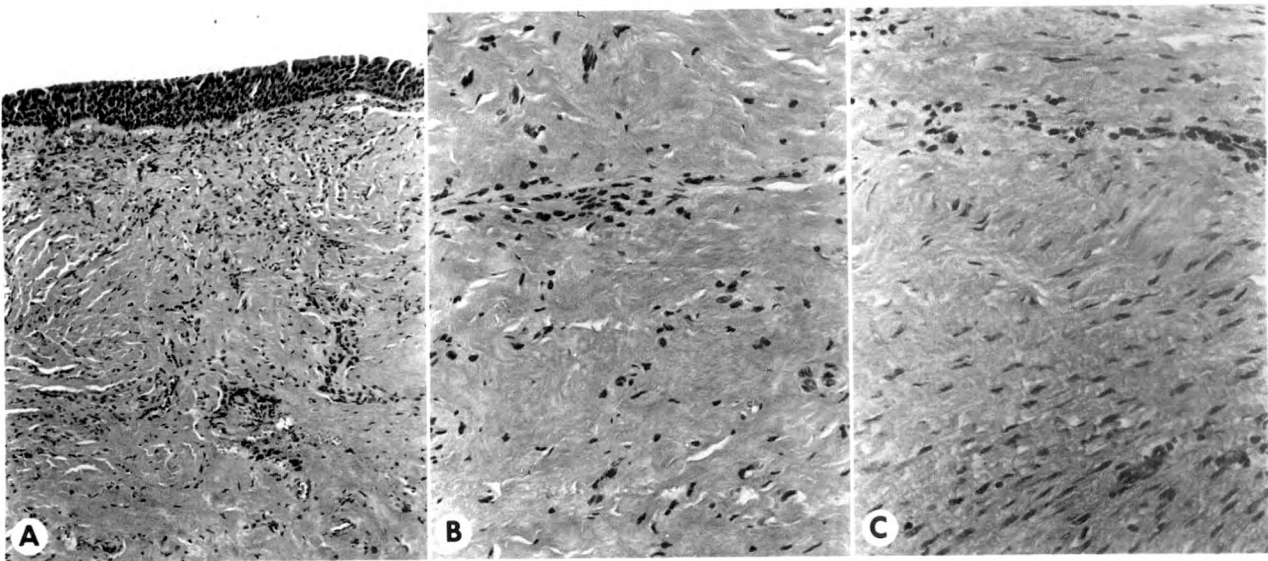


Fig 3. Histopathology of idiopathic subglottic stenosis (H & E). A) Biopsy specimen showing transitional surface mucosa and dense fibrous tissue in lamina propria. Inflammatory cells are sparse. B) Higher magnification of same specimen, showing hypocellular fibrous tissue. C) Another specimen, showing more cellular fibrous tissue resembling fibromatosis.

tracheotomy. Two patients, both treated early in this series, failed laser treatment and were managed with tracheotomy and "permanent" insertion of a hollow, tubular silicone subglottic stent. Both remain tracheotomy-dependent for respiration, but are capable of voice production.

Overall, laser treatment has been satisfactory in 8 of 12 patients (the 11 patients detailed above plus 1 who presented acutely) in whom it was the initial treatment modality. Success in a further patient in the initial stages of treatment is anticipated. None of these patients have dyspnea now, and all rate their voice as normal or near-normal. The mean period of follow-up since last treatment is 28 months (range 3 to 60 months). These patients required an average of 4.2 laser treatments (range 2 to 8), and the average interval between treatments was 2.8 months (range 1 to 12).

DISCUSSION

The most common cause of acquired subglottic stenosis is trauma from prolonged intubation; other causes include external airway injury, granulomatous diseases including Wegener's granulomatosis, tuberculosis, benign and malignant neoplasms, and amyloid disease.

There have been few references to ISS.¹⁻⁵ Attempts have been made to determine the cause of the disorder, and gastroesophageal reflux has been implicated. Gastric acid applied to the subglottis of dogs previously subjected to mucosal injury led to perichondritis, chondritis, and subsequent stenosis.⁶ Jindal et al,⁵ while unable to demonstrate convincing objective evidence of gastroesophageal reflux in 7 patients

with ISS, noted improvement in their patients after empiric therapy with a histamine receptor antagonist. Thirteen of our 15 patients denied symptoms of reflux, and no attempt was made in these patients to objectively exclude "silent" gastroesophageal reflux.

De Vries et al⁷ demonstrated either pANCA or cANCA in 6 patients with isolated subglottic stenosis. In 2 of their patients, a necrotizing granulomatous vasculitis was the cause of subglottic stenosis.

There exists, therefore, a group of patients with subglottic stenosis of no defined cause; our series is presented to further characterize this condition. A history of previous endotracheal intubation in 5 of the patients aroused some suspicion that their stenosis might be a result of intubation trauma, but the brevity of the intubations, the long interval between intubation and onset of their symptoms, and the finding of the characteristic histopathologic appearance of ISS made intubation an unlikely factor.

That this condition appears to occur only in females has not been explained. The larynx is a secondary sex organ, and the presence of sex hormone receptors has been demonstrated in laryngeal and other head and neck anatomic areas.^{12,13} We could not demonstrate estrogen receptors in any of 10 specimens; the negative assays may reflect the low density of hormone-responsive cells or lack of sensitivity of the technique. If estrogen receptors had been found, there might have been therapeutic implications.

The histopathologic appearance suggests that ISS is an inadequately controlled, exuberant fibrotic response, possibly occurring after either trivial or self-limited initiating stimuli. The condition is comparable in appearance with such conditions as abdomi-

nal or extra-abdominal fibromatosis, idiopathic retroperitoneal fibrosis, collagenous colitis, and inflammatory pseudotumors in various body sites. It is of interest that some of these conditions are more common in females, that some are related to prior trauma or infection, that some have a known genetic basis, and that some have responded to hormonal manipulation or the administration of nonsteroidal anti-inflammatory medication.¹⁴

The clinical course of ISS is unpredictable, and it is difficult to treat. Options include close observation, tracheotomy, endoscopic laser surgery, and open surgical procedures, including resection. Our limited experience with laryngotracheoplasty using autolo-

gous costal cartilage grafts has been disappointing. Grillo et al⁴ reported good or excellent results in 32 of 35 selected patients with ISS treated by single-stage resection of stenosis and reconstruction. Those who fail endoscopic surgery should be considered for this procedure.

Although the cause is unknown, it is possible to recognize ISS as an acquired condition with a characteristic endoscopic and histopathologic appearance. It is progressive and potentially life-threatening. Awareness should lead to more ready diagnosis. Our experience indicates that endoscopic laser therapy can be an effective initial primary treatment in many patients.

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SURGICAL CLOSURE OF PERSISTING FAILED TRACHEOESOPHAGEAL VOICE FISTULA

ARIE ROSEN, MD

NEWARK, NEW JERSEY

NATAN SCHER, MD

HARVEY, ILLINOIS

WILLIAM R. PANJE, MD

CHICAGO, ILLINOIS

Tracheoesophageal puncture (TEP) is a highly successful procedure for voice restoration. Occasionally, however, patients fail to achieve satisfactory voice or develop salivary leakage through the fistula into the trachea. Closure of the TEP is then necessary. In most cases, spontaneous closure occurs once the prosthesis has been removed. When the fistula does not close spontaneously, surgical closure is indicated to prevent aspiration and pulmonary complications. We describe a three-layer closure technique that employs interposition of dermal graft. The technique was used on 14 patients over a 7-year period. Most patients received irradiation to the neck. Complete closure was achieved in 13 of 14 cases; 1 patient developed partial breakdown of the closure. Our technique is relatively easy to perform and has a high success rate (92%). Irradiation did not adversely affect the closure rate.

KEY WORDS — fistula, tracheoesophageal fistula, voice.

INTRODUCTION

Speech restoration after total laryngectomy is of great significance for the social and economic welfare of laryngectomy patients. Both esophageal speech and voice restoration with tracheoesophageal puncture (TEP) are superior to the use of an electrolarynx, because they provide a more socially acceptable voice without the need for an electronic device. Unfortunately, approximately 50% of laryngectomy patients are unable to learn esophageal speech.^{1,2} For those patients, TEP is an effective and highly successful technique for voice restoration. Over 80% of patients with a tracheoesophageal voice prosthesis develop intelligible speech.²⁻⁴ Careful patient selection is imperative. Factors such as manual dexterity, level of motivation, ability to maintain the prosthesis, pulmonary reserve, performance on an insufflation test, and adequacy of the stoma should be thoroughly assessed prior to executing TEP.¹ Failure of voice restoration with TEP is frequently a result of poor preoperative patient selection.¹

Leakage of saliva and food through the fistula into the trachea is the most common complication of TEP. Mild saliva and food spillage has been reported in up to 10% of patients with TEP without any concomitant report of adverse consequences. Severe leakage of saliva and food into the trachea may lead to aspiration pneumonia, which can be life-threatening in patients with preexisting chronic lung disease. Most salivary

leakage responds to conservative measures such as temporary reduction of the size of the prosthesis. This allows shrinkage of the fistula tract. Cauterization of the tract has also been used occasionally to promote shrinkage of the fistula.¹

Surgical closure is indicated in the case of an enlarging fistula, or when all nonsurgical measures to reduce the size of the fistula have failed and leakage into the trachea persists.

We describe a three-layer closure technique that we have used on 14 patients. We utilized an interposition dermal graft between a two-layered mucosal closure. This technique does not require a separate skin incision.

TECHNIQUE

The procedure is performed under general anesthesia. A small, flexible endotracheal tube is used to ventilate the patient. A 34F esophageal dilator is then introduced into the esophagus, to elevate the esophageal-tracheal common wall. The area that surrounds the TEP is infiltrated with 1% lidocaine with epinephrine 1:100,000. The size of the infiltrated area should be four to five times the diameter of the fistula. A circumferential circular incision is then made in the tracheal mucosa, 2 to 3 mm lateral to the fistula (Fig 1). The tracheal mucosal circle is undermined toward the center of the fistula, then inverted into the esophageal lumen and sutured with 5-0 interrupted absorbable Vicryl sutures

From the Section of Otolaryngology-Head and Neck Surgery, New Jersey Medical School (University of Medicine and Dentistry of New Jersey), Newark, New Jersey (Rosen), the Department of Otolaryngology, Ingalls Memorial Hospital, Harvey, Illinois (Scher), and the Section of Otolaryngology-Head and Neck Surgery, Rush-Presbyterian Medical Center, Chicago, Illinois (Panje).

CORRESPONDENCE — Arie Rosen, MD, Section of Otolaryngology-Head and Neck Surgery, New Jersey Medical School (UMDNJ), 185 S Orange Ave, H-592, Newark, NJ 07103.

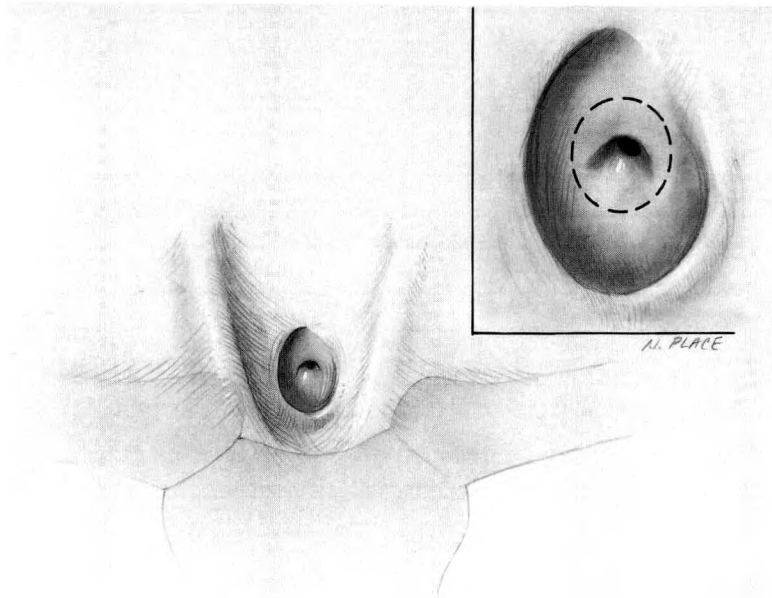


Fig 1. Circumferential circular incision is made in tracheal mucosa.

(Fig 2).

A peripheral tracheal mucosal flap is then created in the previously infiltrated area by undermining the tracheal mucosa centrifugally. A 3×3 -cm thin dermis graft is harvested from the thigh and trimmed to the size of the area of the fistula plus the area of the tracheal mucosal flap. Four to eight equally spaced 4-0 Vicryl sutures are passed through the graft. These sutures are then passed through the tracheal mucosal flap and tied on the tracheal mucosal surface (Fig 3). The last step is to cover the dermal graft by closing the tracheal mucosal layer edges with 4-0 Vicryl sutures.

At the end of the procedure, a size 10F to 12F nasogastric (NG) feeding tube is introduced and left in

place for 7 days. The patient is given intravenous antibiotics during the procedure and kept on oral antibiotics for 7 days thereafter.

RESULTS

Since 1988, 14 patients in four institutions (University of Chicago Hospitals and Ingalls Memorial Hospital in Illinois, East Orange Veterans Administration Medical Center in New Jersey, and San Antonio Veterans Administration Medical Center in Texas) underwent closure of a TEP using the three-layer closure technique. Of the 14 patients, 12 had their fistulas created in outside institutions. They were referred for fistula closure after nonsurgical attempts at closure had failed. A retrospective review of the patients' charts

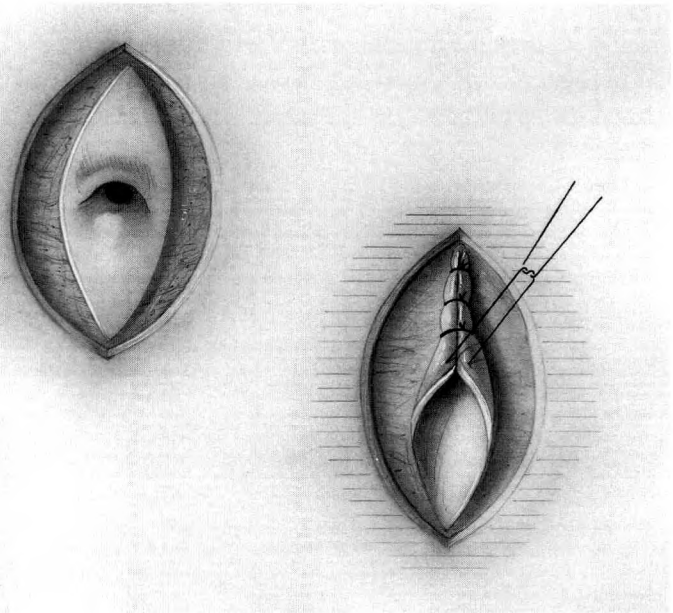
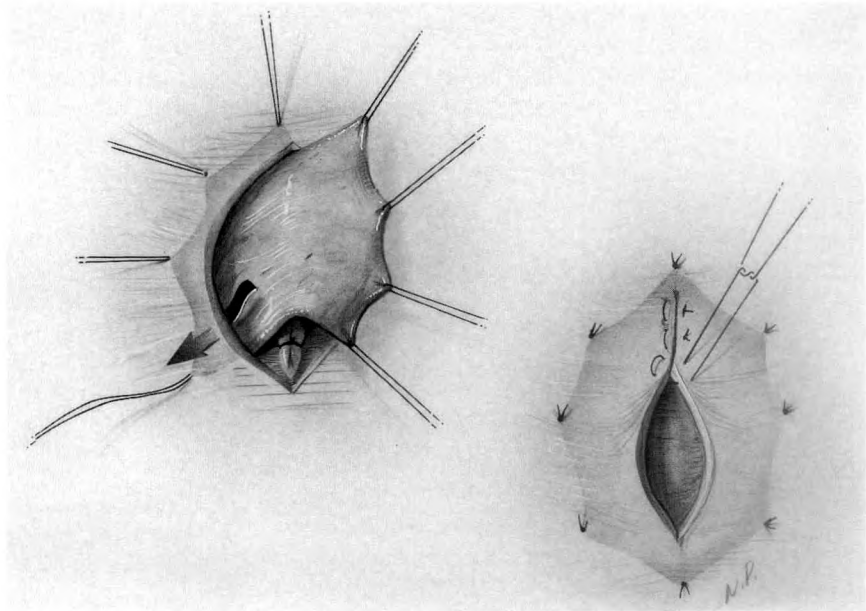


Fig 2. Tracheal mucosal circle is inverted into esophageal lumen and sutured with 5-0 interrupted absorbable Vicryl sutures.

Fig 3. Dermal graft is interposed between trachea and esophagus. Its position is secured with four to eight 4-0 Vicryl sutures that are passed through graft and tracheal mucosal flap and then tied at tracheal side.



revealed that the majority (13) were males. Patients' ages ranged between 48 and 85 years (see Table). All but 1 patient received radiotherapy prior to TEP. The age of the TEP ranged from 3 months to 3 years (median 9 months, mean 14.5 months). All fistulas persisted in spite of nonsurgical efforts to achieve closure (eg, insertion of smaller tubes and cauterization). Leakage of esophageal contents was the indication for closure of the fistula in most cases (8 patients); 4 of the 8 patients developed documented aspiration pneumonia. One patient developed severe emphysema requiring temporary gastrostomy. Six patients failed to acquire voice; 4 of those patients were offered additional treatment

(surgical or medical) to improve their speech, but they preferred to have closure of the fistula and to use an electrolarynx. One patient requested closure of his fistula because of difficulties with its maintenance. The majority of the fistulas had a diameter of 4 to 5 mm; one fistula had a diameter of 6 mm.

Follow-up time ranged from 1 to 48 months with a mean of 21 months. Of 14 patients, 13 (92%) had successful closure of their fistulas. One patient (No. 9) had partial breakdown of the closure; he developed a hematoma at the closure site secondary to a clotting disorder associated with liver cirrhosis. The patient

PATIENT CLINICAL CHARACTERISTICS

Patient No.	Age (y)	Sex	Previous Irradiation Treatment	Age of Fistula (mo)	Indications for Closure	Successful Surgical Closure	Length of Follow-up (mo)
1	52	M	Yes	6	Aspiration pneumonia, patient request	Yes	48
2	68	M	Yes	4	Salivary leak, patient request	Yes	42
3	48	M	Yes	5	Salivary leak, patient request	Yes	30
4	72	M	Yes	24	Aspiration pneumonia	Yes	24
5	51	F	Yes	12	Aspiration pneumonia	Yes	24
6	63	M	Yes	3	Patient request	Yes	24
7	81	M	Yes	4	Failure of speech rehabilitation	Yes	24
8	52	M	Yes	12	Salivary leak	Yes	24
9	67	M	Yes	18	Salivary leak, failure of speech rehabilitation	Partial breakdown	Lost to follow-up
10	85	M	No	36	Failure of speech rehabilitation, emphysema	Yes	18
11	78	M	Yes	4	Failure of speech rehabilitation	Yes	6
12	67	M	Yes	36	Aspiration pneumonia	Yes	4
13	73	M	Yes	3	Failure of speech rehabilitation	Yes	1
14	74	M	Yes	36	Failure of speech rehabilitation	Yes	3

received hyperbaric oxygen treatments, intravenous antibiotics, and intensive wound care; however, the fistula recurred and he was discharged with NG tube feedings. One patient (No. 10) developed ischemic electrocardiographic changes after surgery and stayed in the hospital for 5 days. The majority of the patients (8) were discharged home the same day, and 2 patients were discharged within 24 hours of the TEP closure. With the exception of 1 patient (No. 9), all resumed normal oral intake on postoperative day 7.

DISCUSSION

Various techniques have been proposed for closure of tracheoesophageal fistulas. Most techniques were designed for closure of large fistulas secondary to trauma or other causes.^{5,6} In most cases, muscle (sternocleidomastoid or pectoralis major) is interposed in the tracheoesophageal party wall. Those procedures are not suitable for closure of TEP, because bulky muscle interposed at the TEP area would compromise the tracheal and esophageal lumens.

There is only one previous report of a technique designed specifically for closure of TEP.⁷ In that technique, an interposition dermal graft was placed between the trachea and the esophagus through a separate skin incision just above the tracheal stoma.⁷ Our technique is similar in that we used an interposition dermal graft in a three-layer closure technique, but we did not use a skin incision. Separate skin incision in an irradiated neck has potential for added morbidity. Our technique is relatively easy to perform and does not require a muscle flap.

Most of our patients (13 of 14) received a full course of irradiation to the neck after their laryngectomy. It appears that previous irradiation per se did not affect the closure rate. In spite of the fact that we implanted a dermal graft in an irradiated and saliva-contaminated field, none of our patients developed wound infection, nor was dermal graft take compromised by contamination. All patients received intravenous broad-spectrum antibiotic coverage followed by 7 days of oral antibiotic treatment because of the potential for infection. Most patients were discharged within 24 hours after the operation. For the first few postoperative days, feeding through an NG feeding tube was used to divert food from the closure site while providing adequate nutrition.

Of 14 patients, 9 were referred for closure of their TEP within the first year after its creation. Even though the number of patients is small, one may speculate that fistula failure (due to salivary leak, maintenance failure, or failure to develop speech) is more likely to occur within the first year following the creation of a fistula. A medical complication (aspiration pneumonia) was the primary indication for fistula closure in 4 patients. In 2 of those 4 patients, aspiration pneumonia developed more than a year after the creation of the TEP.

CONCLUSIONS

Closure of tracheoesophageal speech fistulas should be executed in patients who develop pulmonary complications secondary to salivary leakage. It appears that the three-layer closure technique described above is highly successful and has minimal surgical morbidity in spite of previous irradiation.

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RESULTS OF SURGICAL SALVAGE OF LOCOREGIONAL RECURRENCE OF CARCINOMA OF THE TONGUE AFTER RADIOTHERAPY FAILURE

ANTHONY PO WING YUEN, FRCS

WILLIAM IGNACE WEI, FRCS

WAI KUEN HO, FRCS

LAI KUN LAM, FRCS

DORA KWONG, FRCS

HONG KONG, PEOPLE'S REPUBLIC OF CHINA

The aim of this study was to evaluate the efficacy of surgical salvage of local and nodal recurrence of carcinoma of the oral tongue after radiotherapy failure. Of the 47 patients in this retrospective review (between 1980 and 1992), there were 25 with local recurrence alone, 11 with locoregional recurrence, and 11 with nodal recurrence alone. There was no operative mortality, and 17% of patients had one or more surgical complications, including wound infection, flap necrosis, anastomotic leakage, and chest infection. Twenty-nine (62%) patients developed recurrences in the head and neck region after the salvage operation, and 9 of them had second surgical salvage operations. At the last follow-up, 53% of patients had died of carcinoma of the tongue and the overall 5-year actuarial survival rate was 43%. The 5-year actuarial survival rates were 39% for local recurrence alone, 27% for locoregional recurrence, and 68% for nodal recurrence alone. Surgical salvage had satisfactory results for patients with oral tongue carcinoma after radiotherapy failure. Close follow-up and early surgical intervention are essential for patients primarily treated with radiotherapy.

KEY WORDS — carcinoma, glossectomy, radiotherapy, tongue.

INTRODUCTION

Carcinoma of the tongue is a common head and neck cancer. It is primarily treated by surgery, with radiotherapy used as an alternative treatment.¹⁻⁷ The methods of radiotherapy include brachytherapy and external radiotherapy. Due to the high incidence of nodal recurrence even for early-stage carcinoma, a combination of brachytherapy with external radiotherapy to include the neck is often used. Local and nodal recurrences are the commonest sites of treatment failure after radiotherapy. The incidence of locoregional recurrence after radiotherapy is 20% to 85%, depending on the stage and method of radiotherapy.⁴⁻⁶ Surgical salvage for recurrence is the most reasonable treatment of choice after radiotherapy failure. Chemotherapy has little therapeutic value and is usually reserved for palliation. The therapeutic efficacy of surgical salvage is important for patients who choose radiotherapy as their initial treatment; the results are, however, not well documented. The aim of the present review is to provide more information on the results and prognosis of surgical salvage of locoregional recurrence after radiotherapy failure.

PATIENTS AND METHODS

We reviewed the records of patients who had undergone surgical salvage for locoregional recur-

rence after radiotherapy for squamous cell carcinoma of the oral tongue between January 1980 and December 1992. Patients with carcinoma of the tongue base and nonsquamous carcinoma of the tongue were excluded. During this study period, there were 27 patients with carcinoma of the oral tongue (5 stage I, 5 stage II, 3 stage III, 14 stage IV) who received primary radiotherapy treatment in our hospital. Ten patients developed recurrences (7 local, 3 nodal), and 7 of them underwent surgical salvage, including 5 with local recurrences and 2 with nodal recurrences. There were another 40 patients who were referred to our department for surgical salvage after radiotherapy failure in other hospitals. Altogether, there were 47 patients included in the present study of surgical salvage of radiotherapy failure.

The radiotherapy treatment in our hospital was external radiotherapy with lateral opposing facio-cervical fields. A total of 60 to 70 Gy was delivered in 2 to 2.5 Gy per fraction.

The surgical treatment of local recurrence after radiotherapy failure was glossectomy. More extensive resection was sometimes carried out, depending on the sites of involvement, and it might have to include the mandible, tonsils, and larynx. Small defects after resection were closed primarily. Large

From the Departments of Surgery (Yuen, Wei, Lam, Ho) and Radiation Oncology (Kwong), The University of Hong Kong, Queen Mary Hospital, Hong Kong. The study is supported by research grants from The University of Hong Kong (335/048/0040,0067 and 337/048/0009,0012,0014).

CORRESPONDENCE — Anthony Po Wing Yuen, FRCS, Dept of Surgery, Queen Mary Hospital, Pokfulam Road, Hong Kong, People's Republic of China.

TABLE 1. RESULTS OF FIRST SURGICAL SALVAGE

Site of Tumor	No. of Patients	Local Recurrence		Locoregional Recurrence		Regional Recurrence		Distant Metastasis	
		No.	%	No.	%	No.	%	No.	%
Local	25	11	44	1	4	5	20	0	0
Locoregional	11	3	27	1	9	2	18	2	18
Nodal	11	1	9	0	0	5	45	1	9
Total	47	15	32	2	4	12	26	3	6

resection defects were closed with skin grafts in the early period, and more recently were replaced by a pectoralis major myocutaneous flap or free forearm flap. Patients with a clinically node-positive neck had classic radical neck dissection. Elective neck dissection for a node-negative neck was also done when flap reconstruction was necessary.

Locoregional recurrences after the initial surgical salvage were treated with second salvage operations whenever feasible for early recurrence. Patients with more advanced locoregional recurrences and distant metastases who were not surgically salvageable were given palliative treatment. Palliative systemic chemotherapy, mainly consisting of cisplatin and 5-fluorouracil, was considered only in patients who were medically fit for the treatment.

The patients were divided into three groups according to their sites of recurrence: local recurrence alone (group 1), locoregional recurrence (both local and nodal; group 2), and nodal recurrence alone (group 3). The time period from the first salvage operation date to the last follow-up date was used in the calculation of actuarial survival. The life-table method and Wilcoxon (Gehan) statistics were used in the analysis of actuarial survival.

RESULTS

There were 47 patients, including 35 men and 12 women. The median age was 57 years (range 22 to 77 years). They all had radiotherapy failure: 26 (55%) external radiotherapy, 14 (30%) brachytherapy, and

7 (15%) a combination of brachytherapy and external radiotherapy. The preradiotherapy clinical stages were known in 36 patients, including 11 stage I, 12 stage II, 9 stage III, and 4 stage IV patients. Of these 47 patients, there were 25 local recurrences alone (group 1), 11 locoregional recurrences (group 2), and 11 nodal recurrences alone (group 3). Of the 25 group 1 patients with local recurrence alone, their preoperative clinical stages were T1 in 5 patients, T2 in 9, T3 in 7, and T4 in 4. Of the 11 group 2 patients with locoregional recurrence, the stages were T1N1 in 1 patient, T2 in 3 (N1 in 2, N2 in 1), T3 in 6 (N1 in 4, N2 in 2), and T4N3 in 1. Of the 11 group 3 patients with nodal recurrence alone, the preoperative nodal stages were N1 in 4, N2 in 5, and N3 in 2. The mean follow-up of those patients who were still alive at the last follow-up was 68 months.

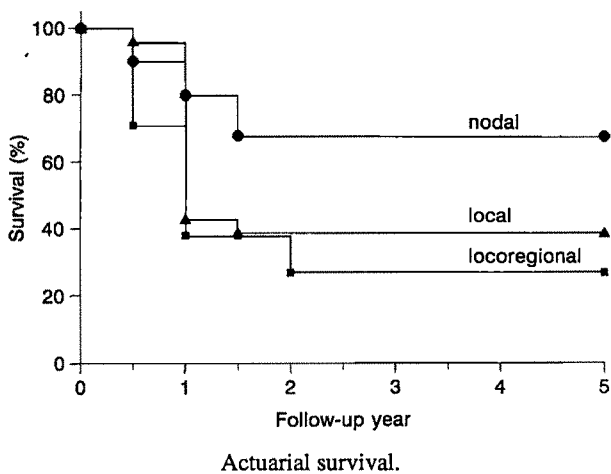
Of the 36 patients who had local recurrence (including local recurrence alone and locoregional recurrence), their salvage operations included 22 partial glossectomy operations, 9 total glossectomies, 3 partial glossectomies with marginal mandibulectomy, and 2 partial glossectomies with segmental mandibulectomy. Regarding reconstruction methods after the local resection, 5 patients had primary closures, 3 skin grafts, 25 pectoralis major myocutaneous flaps, 1 a revascularized free forearm cutaneous flap, and 2 pectoralis major costomyocutaneous flaps. All 22 patients with nodal recurrences (including 11 locoregional recurrences and 11 nodal recurrences alone) had radical neck dissections, including 1 patient with excision of neck skin and deltopectoral flap reconstruction.

There was no operative mortality. Eight (17%) patients had one or more operative complications. There were 4 with wound infection, 3 with partial flap necrosis, 1 with anastomotic leakage, and 1 with chest infection.

The first sites of recurrence after the salvage operations of the three groups of patients are shown in Table 1. Twenty-nine (62%) patients developed recurrences in the head and neck region again after the surgical salvage, and another 3 (6%) patients developed distant metastasis. Of the 36 patients who had surgical salvage for local recurrence (including both

TABLE 2. RESULTS OF SECOND SURGICAL SALVAGE

Site of Recurrence After First Operation	No. of Patients	Second Surgical Salvage		Results of Second Surgical Salvage
		No.	%	
Local	15	3	20	1 died of local recurrence 1 died of nodal recurrence
Locoregional	2	0	0	
Regional	12	6	50	3 died of nodal recurrence
Distant	3	0	0	
Total	32	9	28	5 (56%) died of recurrence



group 1 and group 2), 16 (44%) patients developed local recurrences again (14 local, 2 locoregional). Of the 22 patients (including both group 2 and group 3) who had nodal recurrence with salvage radical neck dissections, there were 8 (36%) regional recurrences again (5 ipsilateral neck, 1 contralateral neck, 1 bilateral neck, 1 local and ipsilateral neck).

Of the 32 patients who developed recurrences again after the first salvage operations, 9 (28%) patients underwent second surgical salvage operations, and their results are shown in Table 2.

In the 12 patients (5 group 1, 2 group 2, 5 group 3) who had regional recurrences after the first salvage operations, there were 11 unilateral neck recurrences, and the prior surgical treatments of these necks were no prior neck dissection (5), radical neck dissection (5), and selective I,II,III neck dissection (1); another 1 patient had bilateral neck recurrence after unilateral radical neck dissection. Six patients with unilateral neck recurrences had second surgical salvage for regional recurrences; they included 4 of the 5 patients without prior neck dissection, 1 of the 5 patients with prior radical neck dissection, and 1 patient with prior selective I,II,III neck dissection. Of these 6 patients with second surgical salvage for neck recurrences, 3 patients subsequently died of neck recurrences.

Of the 9 patients who had second surgical salvage, 5 (56%) patients eventually died of recurrences. All 23 of the other patients (Table 2), who were given palliative treatment, including 1 with brachytherapy for local recurrence and 7 with systemic chemotherapy, died of carcinoma.

At the last follow-up, 25 (53%) patients had died of carcinoma of the tongue, 2 patients had died of other cancer, 6 patients had died of unrelated causes, 1 patient was alive with a second cancer, and 13 patients were alive free of cancer. The overall 5-year disease-free actuarial survival rate of all 47 patients after the first salvage operations was 43%. The actu-

arial survival rates of these three groups of patients are shown in the Figure. The 5-year disease-free actuarial survival rates after surgical salvage were 39% for the group with local recurrence alone (group 1), 27% for locoregional recurrence (group 2), and 68% for nodal recurrence alone (group 3). The pairwise statistical significance levels were $p = .041$ between nodal and locoregional recurrences, $p = .08$ between local and locoregional recurrences, and $p = .188$ between local and nodal recurrences.

DISCUSSION

Radiotherapy is a common treatment option for carcinoma of the oral tongue. Local and nodal recurrences are the most common sites of failure of radiotherapy. Surgical salvage is the most reasonable treatment for recurrence. We have adopted a radical surgical salvage policy for recurrent cancer after radiotherapy, and repeated surgical salvage is often necessary whenever feasible. In our hospital, 70% of patients were candidates for surgical salvage after radiotherapy failure. We have shown that patients who were candidates for the surgical salvage had satisfactory 5-year actuarial survival rates of 27% to 68%, depending on the sites of the recurrence.

Prior irradiation with impaired vascularity and poor wound healing predisposed the patients to wound infection, flap necrosis, and leakage. Despite the 17% complication rate of surgical treatment, it is relatively feasible to perform a major operation after irradiation with no operative mortality.

After the surgical salvage, 62% of patients developed recurrences in the head and neck region again. Distant metastasis only accounted for 6% of the recurrences.

Local recurrence was the most common site of failure after surgical salvage of local recurrence alone. It was, however, found that 20% of patients developed nodal recurrences, and another 4% of patients had locoregional recurrences. Of the 5 patients with nodal recurrences (4 had no neck dissection, and 1 had selective I,II,III neck dissection), 4 patients underwent second surgical salvage operations, and only 1 of them was successfully salvaged. It is known that the incidence of nodal recurrence is high in untreated, clinically node-negative necks.⁸⁻¹⁰ In patients with no prior radiotherapy treatment for carcinoma of the tongue, it is common to give elective treatment to the N0 neck by selective I,II,III neck dissection in the initial surgery.¹¹⁻¹³ In view of the high incidence of nodal recurrence after surgical salvage of local recurrence alone, selective I,II,III neck dissection may also be considered in patients after radiotherapy failure. Its efficacy, however, requires further pro-

spective study.

Locoregional recurrence indicated a more advanced stage of the carcinoma, and was often due to the late detection of recurrence. These patients had a worse prognosis after surgical salvage, with only a 27% 5-year actuarial survival rate compared with a 68% 5-year survival rate for nodal recurrence alone. These patients also had a higher distant metastasis rate: 18%. The poor prognosis of these patients indicates the importance of early diagnosis and surgical intervention for recurrence. Despite the poor prognosis, surgical salvage is the only curative treatment available and is still a worthwhile treatment option for these patients.

Among the 3 groups of patients, those with nodal recurrence alone had the highest survival rate. All 5 subsequent neck recurrences developed on the side of the neck dissection, with 1 patient having bilateral neck recurrence. One patient also developed local recurrence after surgical treatment for nodal recurrence. Of all 5 neck failures, second surgical salvage

for the neck failure was feasible in 1 patient only. Despite the poor secondary salvage result, surgical treatment of nodal recurrence of radiotherapy failure had an overall satisfactory prognosis of 68% 5-year actuarial survival.

Repeated surgical salvage might be necessary, as the initial locoregional failure rate was 62%. Nine of the 32 (28%) patients were candidates for a second surgical salvage for the local and regional recurrences. Although 56% of the 9 patients still died of carcinoma after the second salvage operations, they had a better prognosis compared with those patients who were not candidates for the second surgical salvage. The fact that only early recurrence could be surgically salvaged indicates the importance of early diagnosis of recurrence.

In conclusion, surgical salvage had satisfactory results in the treatment of radiotherapy failure of carcinoma of the oral tongue. It is, however, important to closely follow up the patients in order to have early diagnosis and surgical salvage.

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TRANSORAL TEFLON INJECTION UNDER FLEXIBLE LARYNGOVideosTROBOSCOPY FOR UNILATERAL VOCAL FOLD PARALYSIS

PEN-YUAN CHU, MD

SHYUE-YIH CHANG, MD

TAIPEI, TAIWAN

Intracordal Teflon injection is a widely accepted method for the treatment of glottic incompetence due to unilateral vocal fold paralysis. It can be performed transorally via direct or indirect laryngoscopy, as well as transcutaneously under laryngovideoscopy. However, these procedures still have their disadvantages. The technique we introduce is to perform the procedure transorally while using a curved injection needle under flexible laryngovideoscopic monitoring. With topical anesthesia, the patient can phonate naturally during the procedure. The surgeon can visualize the needle and injection site clearly under high-resolution flexible laryngovideoscopy. Stroboscopic examination during surgery enables the surgeon to monitor the improvement of vocal fold vibration. Twenty consecutive patients treated with this technique were studied. The preliminary results are satisfactory. The technique we introduce is an outpatient procedure with high applicability and low morbidity. This technique is a good, cost-effective alternative to standard operative direct laryngoscopy with injection.

KEY WORDS — Teflon injection, unilateral vocal fold paralysis.

INTRODUCTION

Unilateral vocal fold paralysis frequently results in glottic incompetence. Many surgical techniques have been successfully employed in the treatment of glottic incompetence. These surgical techniques can be classified into three major categories: injection laryngoplasty, laryngeal framework surgery, and laryngeal reinnervation.¹ All of the procedures have advantages and disadvantages.²

Although recent interest in laryngeal framework surgery and reinnervation procedures has prompted a more critical look at injection laryngoplasty, Teflon injection is still a widely accepted method for the treatment of unilateral vocal fold paralysis.³ When Teflon injection is properly performed, especially in the hands of an experienced surgeon, the results are good and complications are limited.⁴ Teflon injection is also the method of choice for many patients with vocal fold paralysis caused by a malignancy with a poor prognosis, or for patients whose medical status makes them poor surgical risks.¹

Teflon injection can be performed transorally via direct laryngoscopy^{5,6} or indirect laryngoscopy,^{7,8} as well as transcutaneously through the cricothyroid membrane under flexible laryngovideoscopy.^{9,10} Injection through a direct laryngoscope can be under local or neuroleptic anesthesia. The procedure is relatively simple, but the patient may experience

more discomfort with this technique. Under direct laryngoscopy, the larynx is distorted and the appearance of the vocal fold is changed. The patient is unable to phonate naturally. The visual and auditory results cannot be precisely monitored during the procedure. Furthermore, the exposure is not always satisfactory in patients with trismus, micrognathia, or cervical spine diseases. With injection under indirect laryngoscopy, the patient can phonate naturally because the larynx is in a physiologic position; however, the technique is relatively difficult in some cases due to a sensitive gag reflex and poor visualization of the larynx.¹⁰ Transcutaneous injection under flexible laryngovideoscopy can be performed with local anesthesia. The patient can phonate naturally during the procedure, and the visual and auditory results can be monitored reliably. The disadvantage of this technique is some surgeons' unfamiliarity with indirect injection of the vocal fold, since the injection needle is inserted to a point under the vocal fold that is not directly visible.¹¹

In this paper, we describe our experience with a new intracordal Teflon injection technique that could overcome the shortcomings of the above-mentioned techniques.

MATERIAL AND METHOD

Between September 1993 and December 1994, 20 consecutive patients with glottic incompetence as a

From the Department of Otolaryngology, Veterans General Hospital, National Yang Ming University, Taipei, Taiwan.

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CORRESPONDENCE — Shyue-Yih Chang, MD, Dept of Otolaryngology, Veterans General Hospital, National Yang Ming University, Taipei, Taiwan.

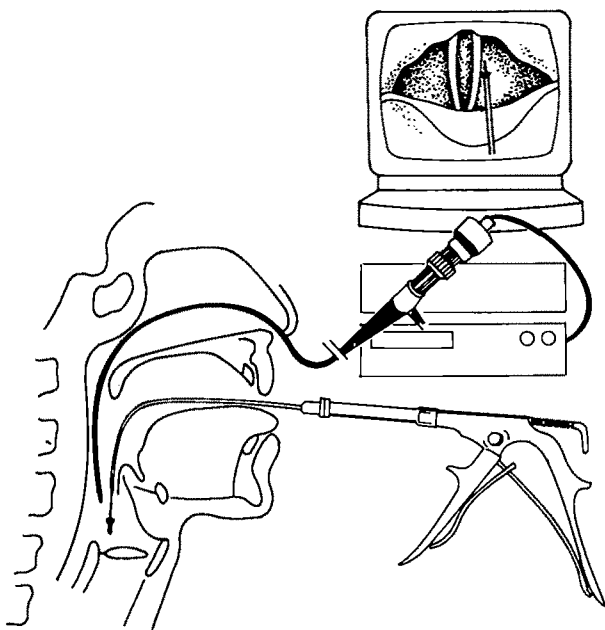


Fig 1. Transoral Teflon injection is performed by means of curved injection needle with visual and auditory monitoring under flexible laryngovideoscopy.

result of unilateral vocal fold paralysis were treated with transoral Teflon injection under flexible laryngovideoscopy. There were no absolute contraindications to use of this technique. Of the 20 patients, 6 were women and 14 were men, and their ages ranged from 20 to 80 years (mean 59 years). The causes of vocal fold paralysis included thyroid surgery, skull base surgery, cardiac surgery, pulmonary and esophageal malignancies, and idiopathic vocal fold paralysis.

The procedure was performed under topical anesthesia at an outpatient office. The patient was seated in a conventional head and neck examination chair. The nose, pharynx, and larynx of the patient were anesthetized with 4% Xylocaine topical spray. Two milliliters of 4% Xylocaine was dripped onto the epiglottis and vocal fold with a curved cannula on a 5-mL syringe. No additional premedication or sedation was necessary. During the procedure, the larynx was visualized with a flexible laryngoscope (Olympus ENF L3) connected to a stroboscopic light source (B&K 4914) and a 3-chip CCD video camera (Hitachi DK-7700). An assistant inserted the flexible laryngoscope and maintained a clear view of the vocal fold on the video monitor. A Bruning syringe was filled with Teflon paste (Ethicon, EH1251) and connected to a curved Rubin-Arnold injection needle with a No. 19 gauge tip and a stop guard 4 mm back from the point. The tongue of the patient was held in place by the surgeon, and the needle was then introduced into the larynx transorally. The vocal fold and the injection needle were clearly visualized on the video mon-

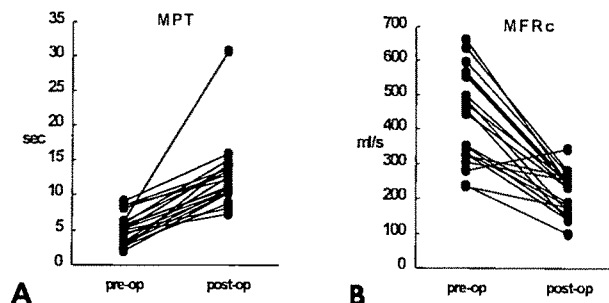


Fig 2. Preoperative and postoperative aerodynamic evaluations. A) Maximum phonation time (MPT). B) Mean airflow rate during comfortable phonation (MFRc).

itor (Fig 1). The first injection was made in the posterior third of the vocal fold, just lateral to the vocal process, and continued until the vocal fold was displaced to the midline. If necessary, a second injection was placed in the middle of the membranous portion of the vocal fold.¹² There was no need to hold the tongue or open the mouth wide during the injection. The improvement of vocal fold vibration and glottic closure was monitored under laryngovideoscopy. The amount of Teflon paste injected depended on the results of visual and auditory monitoring. The injection was ideally completed within 5 minutes.

Preoperative and postoperative evaluations consisted of perceptual voice assessment and aerodynamic analysis, which included maximum phonation time (MPT) and mean airflow rate during comfortable phonation (MFRc). The postoperative perceptual voice evaluation was performed by two otolaryngologists and two speech pathologists. The voice improvement was graded as good, fair, or poor. The voice improvement was considered good if the voice had normal volume with no discernible breathiness or hoarseness. The voice improvement was considered fair if the volume was near normal and there was residual breathiness or hoarseness. The voice improvement was considered poor if the volume was subnormal and breathiness persisted. The postoperative evaluations were performed 2 to 4 weeks after the injection. The patients were also followed up at 6 months after the injection.

RESULTS

Our preliminary experience indicated that the entire procedure can be completed within 20 minutes. In 18 of the 20 consecutive patients, the procedures were performed smoothly. Two patients did not initially complete the injection due to a sensitive gag reflex; however, 1 of them completed the same procedure 2 weeks later. In our study, there was one complication, consisting of local swelling and tenderness of the laryngeal area. The symptoms were

improved after intravenous antibiotic treatment. None of the 20 patients developed airway distress. In the first follow-up at postoperative weeks 2 to 4, the perceptual voice evaluation revealed that 16 of the 20 patients had good improvement of the voice, 3 patients had fair improvement, and 1 patient had poor improvement because of incomplete treatment. Figure 2 shows aerodynamic studies made preoperatively and postoperatively. The MPT increased in all patients following injection. Before injection, the MPT ranged from 1.9 to 9.1 seconds, with an average of 5.2 seconds for all patients. Following injection, the MPT ranged from 7.3 to 30.6 seconds, with an average of 15.0 seconds. Following injection, the MFRc decreased in all patients except 1. The range and average of the MFRc were 233 to 661 mL/s and 433 mL/s preoperatively, and 98 to 342 mL/s and 217 mL/s postoperatively. Six months after injection, 1 patient was lost to follow-up, but the perceptual voice evaluation revealed that 14 of 19 patients had good improvement of the voice, 4 patients had fair improvement, and 1 patient had poor improvement. No obvious deterioration of the voice was noted in our patient population.

DISCUSSION

Although traditional transoral Teflon injection via direct laryngoscopy has been widely accepted, it has disadvantages including considerable discomfort for the patient and limitations in usage due to difficulties with direct laryngoscopy. Functional monitoring during the procedure is also relatively unreliable due to the distorted laryngeal position. Transoral or transcutaneous intracordal Teflon injection under indirect laryngoscopy or flexible laryngovideoscopy can eliminate these obstacles. However, the relative difficulty of the indirect laryngoscopic technique and the unfamiliarity of indirect injection via the transcutaneous technique remain problems. Therefore, we designed a transoral Teflon injection technique guided by flexible laryngovideoscopy.

According to our experience,⁸ the procedure not only decreases the discomfort and limitations of injection under direct laryngoscopy, but also retains the merits of injection in that the patient can phonate naturally, as under indirect laryngoscopy or flexible laryngovideoscopy. The shortcomings of poor visualization of the larynx and a sensitive gag reflex that occur with indirect laryngoscopy were eliminated with this technique.

Although there were no absolute contraindications to the use of our technique, 2 patients failed to complete the procedure during the first attempt. We

believe that in a few patients, it may be difficult to apply the technique due to a hypersensitive gag reflex. However, many surgeons will elect to use the technique due to its convenience and minimal discomfort to the patient. In 18 of our 20 patients, the procedure was performed smoothly and was well tolerated.

In aspects of postoperative phonatory function, our series results are similar to those of a previously published report.¹⁰ This satisfactory functional result can be attributed to the fact that the site, depth, and amount of injection are well adjusted. During the procedure, it is imperative that the injection site and vocal fold vibration be clearly visualized; hence, high-resolution flexible laryngovideoscopy is an essential tool in this technique. In addition, control of the depth of injection is also important for the success of Teflon injection.¹³ In this technique, the judgment of the depth of injection can be facilitated by the stop guard device of the injection needle under video monitoring; difficulty in determining injection depth is a common problem of the transcutaneous procedure. The injection procedure is ideally completed within 5 minutes, as reactive swelling of the vocal fold will interfere with the judgment of the injection amount. The entire treatment can be completed within 20 minutes in an outpatient setting under topical anesthesia; thus, it is not only time-saving, but cost-effective as well.

We believe that overinjection and improper injection can be reduced with our technique under high-resolution video monitoring. Teflon granuloma was not noted in our series, but long-term follow-up is still necessary. Although recent data indicate that Teflon paste injections tend to have a poor long-term result compared to laryngeal framework surgery,¹⁴ this technique remains a good alternative method for patients with vocal fold paralysis caused by malignancies with a poor prognosis and patients whose medical status makes them poor surgical risks.

In comparison with other techniques, this technique has several special requirements: it requires a high-resolution flexible laryngovideoscope; the procedure calls for two specialists, one to give the injection, and the other to maintain the placement of a flexible laryngoscope; and special skill practice is still necessary for the surgeon to master the technique.

In conclusion, transoral Teflon injection under flexible laryngovideoscopy is a highly applicable procedure with low morbidity. It is a good, cost-effective alternative to standard operative direct laryngoscopy with injection.

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HEAD-NECK SURGERY AND ONCOLOGY

The Second Israeli International Symposium on Head-Neck Surgery and Oncology will be held in Jerusalem, Israel, November 1-5, 1998. For more information, please contact the Symposium Secretariat, PO Box 1931, Ramat-Gan 52118, Israel; telephone 972-3-6133340; fax 972-3-6133341.

ROLE OF SUPRAHYOID NECK DISSECTION IN THE TREATMENT OF SQUAMOUS CELL CARCINOMA OF THE LOWER LIP

CAN KOÇ, MD

M. UMUT AKYOL, MD

ALİ ÇEKİÇ, MD

SERDAR ÇELİKKANAT, MD

CAFER ÖZDEM, MD

ANKARA, TURKEY

Treatment of squamous cell carcinoma of the lip is primarily surgical. Unlike other oral lesions, lower lip cancers do not metastasize to lower cervical lymph nodes without invading submental and submandibular lymph nodes. This study presents 30 patients with N0 lower lip carcinoma who were treated by en bloc resection of the tumor with suprahyoid neck dissection. Occult metastasis was found in 4 patients (13%). Four patients, 3 of whom had no occult metastases, died of local or regional uncontrollable disease. Suprahyoid or modified radical neck dissection appears to be beneficial, even in small tumors of the lower lip, in detecting occult metastases.

KEY WORDS — lip cancer, metastasis, neck dissection.

INTRODUCTION

Carcinoma of the lip is the most common malignancy of the oral cavity and occurs most frequently in elderly men.^{1,2} More than 90% of cases occur on the lower lip, and the vast majority are squamous cell carcinoma.² Several etiologic factors are suggested, such as prolonged exposure to sunlight, use of tobacco and alcohol, and chronic immunosuppression.^{3,4} While palpable lymphadenopathy is present in approximately 10% of all patients during the first evaluation, postoperative occult lymphadenopathy seen in the N0 neck ranges from 3% to 9.6%.⁵⁻⁹ Although in some series the 5-year survival rate is almost 100%, the survival rate is poor in cases of cervical metastasis and perineural invasion.^{10,11}

MATERIAL AND METHODS

Forty-seven patients with lower lip squamous cell carcinoma were operated on between 1985 and 1992 at the Ankara Numune Hospital 2nd Otolaryngology Clinic. Patients were classified on the basis of the American Joint Committee TNM classification¹² (Table 1). Clinically, 38 (81%) of the patients had no lymphadenopathy in the neck (N0). Thirty of these patients, 24 men and 6 women, were included in the study, and 8 were excluded because of insufficient follow-up. The follow-up period was at least 3 years for 13 patients, and 5 years for 17 patients. The average age was 54 years (range 36 to 69 years). Three patients had no history of smoking, while the remaining patients had been smokers for 12 to 45 years. The mean period between the recognition of the lesion by the patient and the initial diagnosis was 7 months (range 2 months to 3

years). None of the patients had a history of previous radiotherapy. In addition to excision of the lesion, a bilateral suprahyoid neck dissection (SHND) was performed in 11 patients in whom the lesion invaded the midline, and a unilateral SHND was performed in the remaining 19 patients. Radical neck dissection (RND) was performed on 4 patients who had occult metastases. The operative defects were closed primarily in 5 patients, whereas in 25 cases, Bernard plasty was employed for reconstruction.

RESULTS

Ipsilateral occult metastases were found in 4 of 30 patients (13%). One metastatic lymph node was found in the submandibular region in 2 patients, two metastatic nodes were found in the submandibular region in 1 patient, and three nodes (two in the submandibular region and one in the submental region) were found in the fourth patient. Radical neck dissection was performed on the above-mentioned patients, and no metastases were detected.

Four patients died of disease within 2 postoperative years. One of these patients had undergone RND, since occult metastases had been detected. No metastases

TABLE 1. T CLASSIFICATION OF PATIENTS (N0, M0)

	No.	%
T1	9	30
T2	13	43
T3	8	27
T4	0	0
Total	30	100

From the 2nd Otolaryngology Clinic, Ankara Numune Hospital (Koç, Çelikkanat, Çekiç, Özdem), and the Department of Otorhinolaryngology, Hacettepe University Medical School (Akyol), Ankara, Turkey.

CORRESPONDENCE — Can Koç, MD, Hatir sok. 15/2, GOP Ankara, 06700 Turkey.

TABLE 2. PRESENCE OF OCCULT METASTASES AND SURVIVAL IN PATIENTS GROUPED ACCORDING TO SIZE OF SQUAMOUS CELL CARCINOMA OF LOWER LIP

Tumor Size (T)	No. of Pts.	Occult Metastases	Died
T1	9	(+) 1 (-) 8	
T2	13	(+) 1 (-) 12	1
T3	8	(+) 2 (-) 6	1 2
Total	30	(+) 13.3%	13.3%

were found in the RND specimen. Despite the fact that no occult metastases were detected in the SHND specimens of the remaining 3 patients, neck metastases (contralateral in 1 case) were subsequently observed in all. These patients underwent RND followed by radiotherapy. Table 2 illustrates the numbers and rates of occult metastases, and the data related to the 4 patients who died of disease.

DISCUSSION

Lower lip carcinomas have the best prognosis of oral cavity cancers¹; however, the death of 10 patients in a series³ of 37 is an example of the high mortality of these tumors, necessitating adequate treatment. Similarly, 4 of 30 patients died of the disease in our study. In 3 of these patients, no occult metastases were detected in the histologic examination of SHND. In 1 of these patients, who had a T2 tumor with submucosal invasion close to the midline, no occult metastases were found in the ipsilateral SHND specimen, but in the fourth postoperative month, contralateral neck metastases were observed. This patient had radiotherapy after RND and lived free of disease for 4 years. This case points to the fact that tumors situated in or close to the midline require bilateral neck treatment. A possible explanation for the appearance of neck metastases in 3 patients without occult metastases on SHND can be a lack of careful and detailed histopathologic dissection and examination of the specimens by the pathologists. It can also be due to inadequate surgical technique of the SHND in a teaching hospital clinic where most of the operations are performed by residents. However, this explanation seems unlikely, since neck metastases in all 3 patients were below the SHND limit. Close follow-up and early treatment of local or regional disease is of utmost importance. None of these 3 patients submitted to follow-up examinations, and they returned with advanced neck disease 4, 5, and 6 months after their operations.

Baker and Krause¹³ reported occult metastases in

less than 10% of patients with carcinoma of the lower lip, and there was no difference in survival rates between patients with prophylactic versus therapeutic neck dissection; hence, they oppose neck dissection in N0 necks. Ashley et al¹⁴ concluded that for most lesions limited to the lip, simple V excision is an adequate treatment; however, it has also been reported that lymph node metastasis appearing after simple V excision worsens the prognosis.^{3,7}

We think that once local and regional control of disease cannot be achieved, the prognosis worsens and a salvage treatment is usually not possible, so the initial treatment must be adequate. Even with N0 specimens, careful and detailed preoperative physical and radiologic evaluation of the patient and postoperative collaboration with the pathologist is essential.

Marshall and Edgerton³ recommend bilateral SHND as a biopsy technique in N0 necks if the primary tumor is greater than 1.5 cm, and RND if the suprahyoid biopsy proves positive. In contrast, Baker and Krause¹³ report that tumors less than 2 cm in diameter have an excellent prognosis, with no cervical metastases occurring during the follow-up of tumors less than 1 cm in 85 cases. Ashley et al¹⁴ report that 3 of 15 patients with neck metastases had tumors less than 1 cm in diameter.

The depth of infiltration is an important prognostic factor. Frierson and Cooper¹⁵ reported that lower lip squamous cell carcinomas with metastases to the neck invade three times deeper than nonmetastasizing carcinomas, and the metastasized tumors ranged in size from 2 to 40 mm. Schreiner and Mattick¹⁶ reported 13% nodal metastases in tumors less than 1.5 cm, while Hosai et al¹⁷ stated that primary tumor size did not correlate with the rate of nodal metastases. The presence of occult metastases in 4 of our 30 patients shows the importance of SHND.

Wurman et al¹⁰ recommend prophylactic SHND in patients who have a primary lesion larger than 2 cm without cervical metastases and who appear unlikely to submit to follow-up examinations.

Despite the contrary views in the literature, our study revealed the following.

1. Even in tumors smaller than 2 cm in diameter, an SHND is beneficial, since it has a low morbidity and it excludes the possible risks of a wait-and-see policy. An RND must be performed in patients with metastases revealed by careful histopathologic examination of the SHND.

2. In patients with tumors situated at or close to the midline, bilateral SHND should be performed.

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NEONATAL HEARING SCREENING

The European Consensus Development Conference on Neonatal Hearing Screening will be held in Milan, Italy, May 15-16, 1998. For more information, contact Dr F. Grandori, Centre of Biomedical Engineering, Polytechnic of Milan, Piazza Leonardo da Vinci 32, 20133 Milan, Italy; fax +39-2-23993360; email ecdc@elet.polimi.it.

CORRELATION OF NEUTROPHIL ACTIVATION AND SKIN FLAP SURVIVAL IN PHARMACOLOGICALLY ALTERED PIGS

JAMES R. KOSKO, MD

ORLANDO, FLORIDA

PATRICIA B. WILLIAMS, PHD

NORFOLK, VIRGINIA

MICHAEL F. PRATT, MD

ATLANTA, GEORGIA

In inflamed tissue, neutrophils produce tissue necrosis factors such as free oxygen radicals. We investigated the role of neutrophils in random flap survival using the tissue neutrophil marker myeloperoxidase (MPO), and in whole blood using flow cytometry with the neutrophil activation marker 2'7' dichlorofluorescein diacetate. Hypopigmented pigs were treated with the experimental 21-aminosteroid lipid antioxidant U-74389G (oxygen free radical scavenger) before dorsal random skin flaps were elevated. Extent of flap survival was measured by surface planimetry 7 days after surgery. Mean flap survival was $64.1\% \pm 3.4\%$ in the 3-mg/kg-treated group, and $68.0\% \pm 3.4\%$ in the 1-mg/kg-treated group — both significantly greater than the survival in vehicle-treated controls ($48.6\% \pm 2.3\%$). We measured MPO in tissue extracts using an enzyme-linked immunoassay, which showed less MPO in treated animals than in controls. Flow cytometry results were nonspecific. These data suggest that U-74389G improves random skin flap viability by inhibiting neutrophil infiltration into the flap.

KEY WORDS — flap survival, neutrophil activation, skin flap.

INTRODUCTION

Random skin flaps are often used to repair cutaneous defects. Several animal models have been developed; however, the pig's skin is the most representative of human skin.¹⁻³

An objective method of evaluating random skin flap survival is extremely desirable. In previous pig flap studies, enhancement of survival was noted with the use of several pharmacologic agents.^{4,5} In addition to obvious gross pathologic correlation, several objective parameters, including platelet aggregation, erythrocyte flexibility, and serum fibrinogen levels, were evaluated in these studies. Another objective measure of flap survival is neutrophil activation, which results in the release of injurious metabolites shown to cause local tissue damage.⁶⁻⁹ Evaluation of neutrophil activation includes the measurement of intracellular oxidant production and oxidative activity in whole blood (measured by flow cytometry), as well as tissue neutrophil content measured with the neutrophil marker myeloperoxidase (MPO).^{10,11} Previous studies have demonstrated decreased neutrophil activity after treatment with pharmacologic agents.¹⁰⁻¹²

The goal of this study was to produce an animal model that correlates skin flap survival with neutro-

phil activation. In addition, neutrophil activation was correlated with specific pharmacologic treatments. The 21-aminosteroid U-74389G, and its properties relating to alteration of granulocyte function and improvement of flap survival, was studied in detail.

MATERIALS AND METHODS

Experimental Model. Hypopigmented piglets weighing 20 to 22 kg were used. The animals were housed individually and fed pig chow three times each day. U-74389G, a 21-aminosteroid, was delivered 1 hour before surgery as a bolus via indwelling central venous catheter or percutaneous central venous puncture.¹³ The vehicle for U-74389G was Upjohn CS-4, consisting of 0.02 mol/L citric acid monohydrate, 0.0032 mol/L sodium citrate dihydrate, and 0.077 mol/L sodium chloride, pH 3.0. Control animals received vehicle alone.

Operative Procedures. Six dorsal random skin flaps (three on each side) were elevated on each animal under general anesthesia induced with ketamine hydrochloride (15 mg/kg intramuscularly) and acepromazine maleate (1.1 mg/kg intramuscularly) and maintained with isoflurane (1.5 to 2.0 vol%) after endotracheal intubation. Each flap measured 4 × 14 cm, had its base located 4 cm from the dorsal midline,

From the Department of Otolaryngology—Head and Neck Surgery, Eastern Virginia Medical School, Norfolk, Virginia. Dr Kosko is currently in the Division of Pediatric Otolaryngology, The Nemours Children's Clinic, Orlando, Florida. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Eastern Virginia Medical School.

CORRESPONDENCE — Michael F. Pratt, MD, Atlanta Ear, Nose and Throat Associates, 5555 Peachtree Dunwoody Rd, Suite 235, Atlanta, GA 30341.

and was elevated in the subcutaneous tissue plane superficial to the panniculus carnosus according to the method of Kerrigan and Daniel.¹⁴ After elevation, flaps were closed with interrupted 4.0 Vicryl subcutaneous sutures and 2-0 Supramid skin sutures. All flaps were dressed with nitrofurazone (0.2%) to prevent infection. Percentage of necrosis was determined for each flap by surface planimetry. After measuring surface survival, we euthanized the animals with an overdose of pentobarbital sodium.

Blood Sampling. Samples were drawn before drug treatment, on the day of operation immediately after treatment, 3 days after operation, and on the day of sacrifice via an indwelling internal jugular central venous line.

Tissue Harvesting. Tissue samples weighing approximately 0.2 g were harvested from the following areas: rump, 1 cm from the proximal margin on the flap, and 1 cm from the distal margin on the flap. Samples were taken both 24 hours and 7 days after operation. In addition, several samples were taken preoperatively from the rump area. These samples from the nonoperative area (rump) were used to determine baseline control values.

Detection of Granulocyte Activation in Whole Blood by Flow Cytometry. Neutrophil activation was evaluated by staining with 2'7' dichlorofluorescein diacetate (DCFH-DA, Molecular Probes, Eugene, Ore).¹² This stain measures granulocyte intracellular reactive oxygen production, a marker for granulocyte activation.

Heparinized whole blood samples were obtained from both treated and untreated animals as originally described by Himmelfarb et al.¹² For in vitro studies, U-74389G was added to whole blood from untreated pigs. After incubation at 37°C with agitation, aliquots were diluted (1:8.5) with phosphate-buffered saline. Following the addition of DCFH-DA (5 μ mol/L), samples were incubated at 37°C for 15 minutes before stimulation with standard neutrophil agonists: phorbol myristic acid (PMA), *Staphylococcus aureus*, or zymosan-activated serum at 37°C for an additional 30 minutes. Erythrocytes were lysed with ammonium chloride. Following centrifugation at 250g for 2 minutes, the supernatant was discarded and the cells were reconstituted in phosphate-buffered saline.

Neutrophil activity was then analyzed with the Coulter flow cytometer (Coulter Corp, Hialeah, Fla). In order to identify individual cell populations, the cytometer was calibrated to measure linear forward scatter and linear light scatter. The neutrophil population was identified without difficulty. This popula-

tion was then analyzed for fluorescence at 535 nmol/L, which corresponds to the fluorescein marker present in DCFH-DA. Results were obtained both for mean channel fluorescence, which corresponds to the extent of activation of individual neutrophils, and for percent of total neutrophils activated.

Detection of Myeloperoxidase Activity. Tissue samples (0.2 g) taken from viable and nonviable flap tissue were homogenized by a method described by Bradley et al.¹⁰ Tissues were sliced, minced, and homogenized in 0.5% hexadecyltrimethylammonium bromide (HTAB; Sigma Chemical Co, St Louis, Mo) in potassium phosphate buffer, pH 6.0, before sonication. After three cycles of freeze-thawing, sonication was repeated. Following centrifugation at 40,000g for 15 minutes, the supernatant was assayed by an enzyme-linked immunoassay (MPO-EIA, Bioxytech, Donneuil-Marne, France). Aliquots of the homogenate were incubated in wells containing a monoclonal antibody to MPO at 37°C for 1 hour. Anti-MPO was added to each well and incubated at 37°C for 1 hour followed by avidin-coupled alkaline phosphatase solution at 37°C for 1 hour. To produce a colorimetrically evaluable solution, 4-nitrophenyl-phosphate was added to a diluting buffer of 1 mol/L diethanolamine at pH 9.8. Twenty-five minutes later, absorbance was read at 405 nmol/L.

Statistical Analysis of Data. Data were analyzed with either a one-way or two-way analysis of variance as appropriate. Significant differences were identified with a standard Neuman-Keul's test. Data are expressed as the mean $\pm$ SEM; N represents the number of replications.

RESULTS

Flap Survival. Representative flaps from pigs treated with either vehicle alone (control) or U-74389G (1 mg/kg) 1 hour before operation are displayed in Fig 1. One week after operation, flap survival was significantly greater in pigs treated with U-74389G (64.12% $\pm$ 3.4% in the 3-mg/kg group; 68.04% $\pm$ 3.4% in the 1-mg/kg group) than in vehicle-treated controls (48.66% $\pm$ 2.3%) (Fig 2).

Neutrophil Activation (Measured by Flow Cytometry). The fluorescent signal from the neutrophil population in blood was easily separated from the appearance of other cells. There was no statistically significant difference between samples stimulated with PMA, *S aureus*, or zymosan-activated serum when analyzed at 535 nmol/L for DCFH-DA. Porcine neutrophils were easily stimulated with PMA, thereby producing reliable, reproducible results, while some difficulty was noted with the use of *S aureus* and zymosan-activated serum.

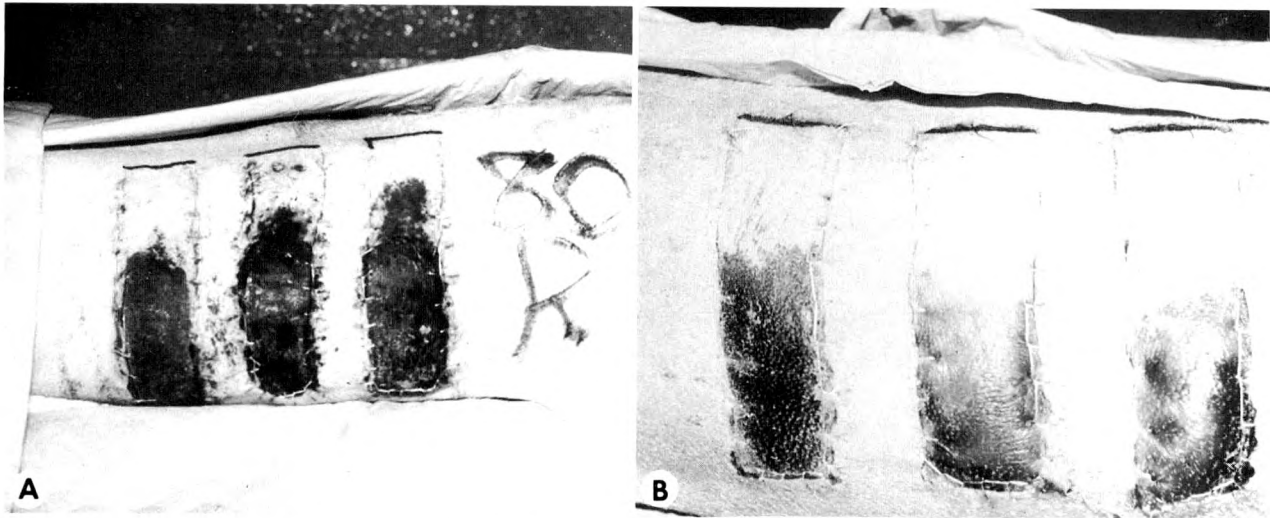


Fig 1. Appearance of skin flaps on seventh postoperative day. A) In vehicle-treated control animal. B) In experimental animal treated with U-74389G (1 mg/kg). Note significant improvement in flap viability when compared with control animal.

The intensities of neutrophil activation by PMA in blood from U-74389G-treated and control pigs were similar (Fig 3). The addition of U-74389G to whole blood did not change the pattern of neutrophil activation. Thus, we were unable to demonstrate any statistically significant correlation between mean channel fluorescence and percent neutrophil activation either in vivo or in vitro with the 21-aminosteroid in various concentrations. In addition, there was near-complete stimulation of all neutrophils with PMA in all experimental subjects.

Neutrophil Activation (Measured by MPO Activity). We expressed MPO as a ratio of the concentration in viable or nonviable flap tissue to the concentration in nonoperated rump (control) tissue. In the tissue from vehicle-treated pigs there was more MPO activity in both viable and nonviable flap tissue than in tissue taken from a nonoperated site (rump; see Table). There was more MPO in necrotic portions of

flaps from U-74389G-treated pigs than in their nonoperated sites (rump). However, in viable portions of flaps from U-74389G-treated pigs there was less MPO than in their nonoperated sites (rump). The amount of MPO from viable tissue in treated pigs was significantly less than the amount in viable tissue from vehicle-treated pigs. Likewise, there was significantly less MPO in viable tissue than in nonviable tissue from the same pigs.

DISCUSSION

Failing skin flaps remain a difficult problem for reconstructive surgeons. Accurate ways of predicting skin flap survival are necessary. Neutrophils have been noted to play a major role in local tissue damage, and hence we sought to identify their role in local skin flap failure. In this study, we investigated activated neutrophils in two different ways. Neutrophil function was investigated systemically by flow cytometry, and locally, by the neutrophil marker MPO, after treatment with the 21-aminosteroid U-74389G (a lazard). Lazardoids have been demonstrated to inhibit superoxide (O_2^-)-induced neutrophil adhesion in a hamster model.¹⁵ In addition, they inhibit hydrogen peroxide production in activated neutrophils.¹⁶

Shin et al¹⁷ have investigated the effect of the 21-aminosteroid U-74389F on skin flap survival after secondary ischemia in a rat model. Myeloperoxidase activity was decreased in the lazard-treated rats. Since MPO is highly specific as a marker for myeloid cells, primarily neutrophils, these results suggest that U-74389F inhibited neutrophil activity in this rat model.¹⁸

Liu et al¹⁹ investigated the 21-aminosteroid tirilazad mesylate, in an experimental rat ischemia-reperfu-

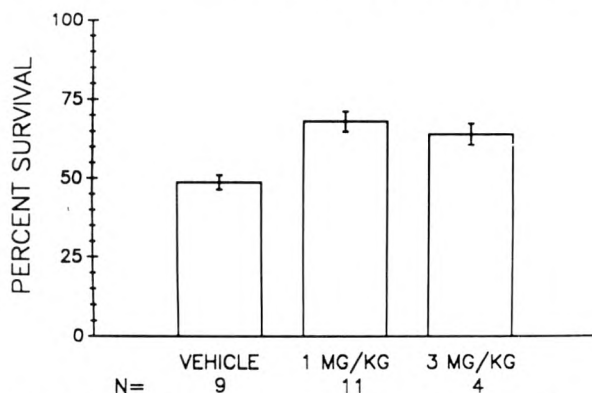


Fig 2. Bar graph showing statistically significant ($p < .05$) improvement in skin flap viability with use of U-74389G in doses of 1 mg/kg and 3 mg/kg (mean survival $64.12\% \pm 3.4\%$) when compared to vehicle-treated controls ($48.66\% \pm 2.3\%$).

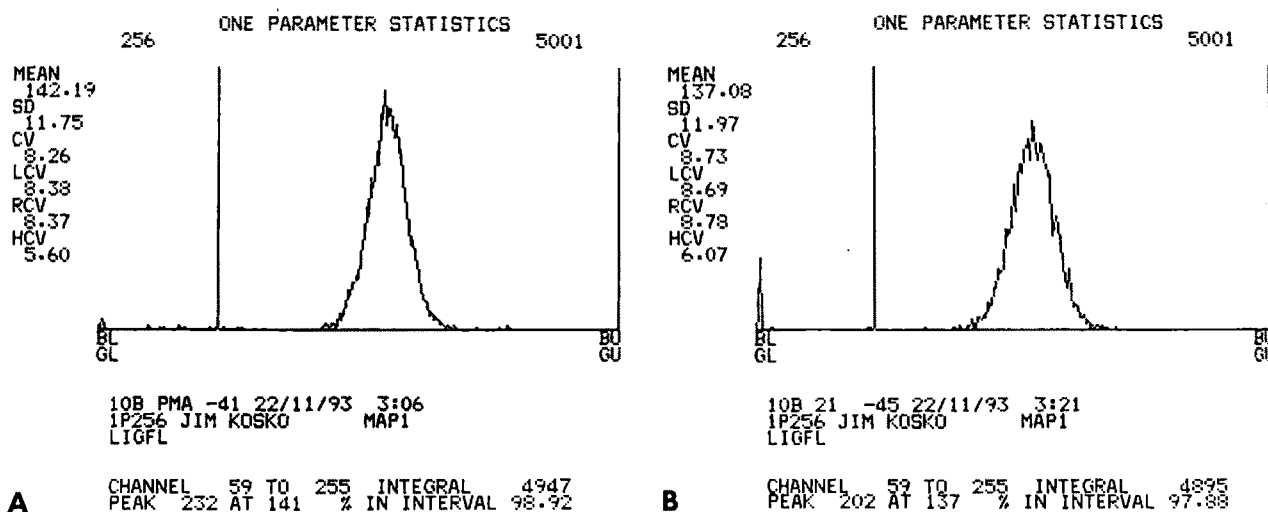


Fig 3. Pattern of neutrophil activation in whole blood samples. A) Samples stimulated with 10 nmol of phorbol myristic acid after staining with molecular probe, 2',7' dichlorofluorescein diacetate. Cells were analyzed for fluorescence at 535 nm/L in Coulter flow cytometer. Mean channel fluorescence, which corresponds to degree of neutrophil activation, was 142.19 nm. B) Samples with U-74389G additive (3 mg/kg). Mean channel fluorescence (137.08 nm) was not statistically different from that of control samples (142.19 nm; shown in A).

sion model of endotoxin shock and acute liver failure. They concluded that the 21-aminosteroid inhibited early tissue injury through inhibition of lipid peroxidation and/or membrane stabilization. In addition, they found 70% less neutrophil infiltration at 48 hours.

Liu et al¹⁹ also reported that tirilazad mesylate does not decrease neutrophil activation in PMA-stimulated neutrophils either in vivo or in vitro. Our findings confirm their results. These data reflect low sensitivity and specificity of the stimulant (PMA), making invalid any conclusions about the 21-aminosteroid effect.

Ovize et al²⁰ studied inhibition of in vivo lipid peroxidation in a canine coronary ischemia reperfusion model by the 21-aminosteroid U-74006F. While they demonstrated decreased lipid peroxidation, a role mediated by neutrophils, they concluded that this was not a consequence of altered neutrophil function. This conclusion was based on the finding that there was no change in peripheral neutrophil counts.²⁰

Although our data conflict with some results of other studies, there is agreement on several key issues. In all studies, neutrophil activation has been altered by a 21-aminosteroid and objectively mea-

sured. In our study, decreased MPO activity (as an index of decreased neutrophil infiltration) correlated with increased flap survival. In other studies, decreased neutrophil activation was measured by decreased in vivo lipid peroxidation and neutrophil infiltration rather than decreased MPO activity, which we found to be a more representative and reliable indicator.

We did not find the measurement of activated circulating neutrophils to be an accurate predictor of the extent of flap survival or the degree of local neutrophil activation. We were unable to measure any significant differences in the number of activated neutrophils between treated and control pigs. In addition, it stands to reason that activated neutrophils are present at the region of local tissue damage and should not be found in the circulation to any significant degree. A previous study similarly demonstrated poor correlation between circulating neutrophils and local tissue changes.²⁰

There are several limitations of this study. The two most obvious are the choice of neutrophil stimulant and our initial emphasis on systemic neutrophil activation. Originally, we investigated the correlation between circulating activated neutrophils and skin flap survival. The PMA stimulation of neutrophils was the only reproducible technique of those tested. Additionally, PMA-stimulated neutrophil activation was an all-or-nothing event — a fact previously discovered by others.¹⁸ Thus, we found that drug stimulation of activated circulating neutrophils lacks sensitivity and specificity as a marker for local tissue damage.

MYELOPEROXIDASE TISSUE CONCENTRATIONS

Treatment	Viable	Necrotic	N
Control	1.30 ± 0.25	1.49 ± 0.25	4
U-74389G (3 mg/kg)	0.68 ± 0.08*	1.10 ± 0.09	3

Myeloperoxidase is expressed as ratio (concentration in viable or necrotic tissue sample over concentration in nonoperated tissue sample).

*p < .05.

Techniques different from PMA stimulation are available. Analysis of our data suggests that measurement of tissue MPO levels is a more sensitive indicator of neutrophil activation and local damage from the resultant oxygen free radical production. Consequently, future studies should be directed at further investigating the correlation between tissue MPO levels and tissue survival. We are currently studying tissue neutrophil infiltration by histopathologic analysis. Additionally, we are presently investigating the correlation between flap survival and tissue lipid peroxidase levels by a new technique. Lipid peroxidase is another marker for neutrophil activation.

Once current and future methods are tested and refined, therapeutic intervention based on sound pathophysiologic and pharmacologic principles can be established to enhance the survival of skin flaps used in reconstructive surgery. This work will be instrumental in the development of an animal model that can be used in future studies to reliably measure local tissue interactions that are reflective of and predictive for early skin flap necrosis.

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SUMMARY

Our overall objective was to design an animal model in which percentage of neutrophil activation is an indicator for skin flap survival. A secondary goal was to show a correlation between pharmacologic treatment and tissue neutrophil activation. This study demonstrates a direct correlation between improved skin flap survival and the use of an experimental 21-aminosteroid antioxidant.

It is well known that injurious metabolites (such as oxygen free radicals) released by activated neutrophils result in local tissue damage. It is therefore desirable to obtain an objective method of evaluating neutrophil activation as a measure of tissue necrosis. In this study, we have demonstrated a correlation between a tissue marker for activated neutrophils and poor skin flap viability using a monoclonal antibody for measurement of MPO levels. Further investigation should focus on the dose response for multiple skin flap-enhancing drugs and eventual human testing, after measurement of markers for activated tissue neutrophils is further refined in the laboratory.

IMAGING CASE STUDY OF THE MONTH

MAGNETIC RESONANCE IMAGING OF VOCAL FAILURE IN PATIENTS UNDERGOING LARYNGECTOMY WITH TRACHEOESOPHAGEAL FISTULA

HIROBUMI KUMAZAWA, MD

YOSHINAO TSUTA, MD

TOSHIO YAMASHITA, MD

AKIHIKO NAKAMURA, MD

HIROAKI KUROKAWA, MD

OSAKA, JAPAN

INTRODUCTION

The restoration of speech is the ultimate goal in patients who have undergone a laryngectomy. Surgical voice restoration using the Blom-Singer voice prosthesis is a well-established procedure, and has been reported to yield favorable results with a valved, silicone rubber voice prosthesis inserted in a tracheoesophageal fistula (TEF).¹ However, with increasing experience, including that at our clinic,² it has become apparent that this relatively simple procedure is not totally benign. Most patients seem to have a variety of minor and/or major complications, as has been previously reported.²⁻⁷

In this report, we describe the magnetic resonance imaging (MRI) of eight postoperative patients with laryngeal cancer. Although the eight patients achieved satisfactory voice characteristics by surgical restoration using the Blom-Singer voice prosthesis, two of the patients complained of difficult vocal production during long-term follow-up after laryngectomy. The causes of their vocal failure are discussed by analyzing their MRI features.

PATIENTS AND METHODS

Eight laryngeal cancer patients, ranging in age from 51 to 74 years old, were studied by MRI. All of the patients underwent a total laryngectomy and TEF with the Blom-Singer voice prosthesis from January 1983 to December 1991 at the Department of Otolaryngology, Kansai Medical University. All patients have been followed up for a minimum period of 9 months after achieving a satisfactory voice by surgical voice restoration. The characteristics of these patients are shown in the Table. Two of the eight

patients complained of vocal failure during the observation period, but the other patients had no problems with their vocal function.

All of the studies were performed with a 1.5-T MRI scanner (MRT200 IxIII; Toshiba, Tokyo, Japan). The studies included conventional spin-echo T1-weighted (TR/TE/NEX, 500-700/15/2-4) and T2-weighted (TR/TE/NEX, 2,500-4,000/80-95/1-2) images in the axial, coronal, and sagittal planes. The section thickness was 5 to 7 mm with a 0.5- to 1-mm intersection gap. The field of view was 20 cm, and the

CHARACTERISTICS OF PATIENTS
UNDERGOING LARYNGECTOMY WITH
TRACHEOESOPHAGEAL FISTULA

Patient No.	Age	Sex	TNM (Stage)	Problems With Prosthesis	Observation Time (mo)	Time of Vocal Failure (mo)
1	71	M	T2N1M0 (III)	No	100	
2	51	M	T2N0M0 (II)	Yes		47
3	55	F	T2N1M0 (III)	No	57	
4	63	M	T4N0M0 (IV)	No	73	
5	74	M	T2N0M0 (II)	No	9	
6	62	M	T3N1M0 (III)	No	114	
7	59	M	T2N2cM0 (III)	Yes		66
8	74	M	T2N0M0 (II)	No	29	

From the Departments of Otolaryngology (Kumazawa, Tsuta, Nakamura, Yamashita) and Radiology (Kurokawa), Kansai Medical University, Osaka, Japan.

CORRESPONDENCE—Hirobumi Kumazawa, MD, Dept of Otolaryngology, Kansai Medical University, Fumizonochō 10-15, Moriguchi City, 570 Osaka, Japan.

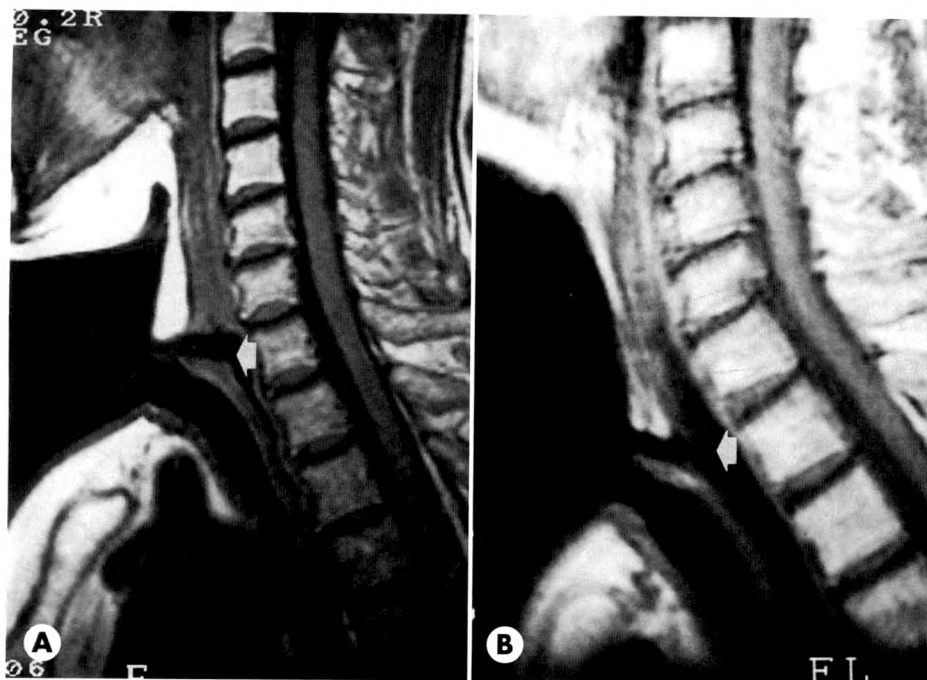


Fig 1. Lateral views of magnetic resonance images of patients with satisfactory voice. Arrows — air lumen of esophagus, which was located at level of fistula. A) Case 3. B) Case 4.

image matrix was 256×256 . The patients were examined during normal respiration without speech. Salivary leakage and sputa at the site of TEF were suctioned by a suction tube before the examination.

RESULTS

Patients With Satisfactory Voice Characteristics. Six of the eight patients (Nos. 1, 3, 4, 5, 6, and 8) continued to have a satisfactory voice after the surgical voice restoration using a Blom-Singer voice prosthesis. The MRI showed a fistula located from the upper part of the trachea to the esophageal space.

Four of the six patients with a good voice (Nos. 3-6) demonstrated the utility of MRI in identifying the air space of the esophagus, which was located at the level of the fistula. The tent shape due to the Blom-Singer voice prosthesis inserted into the fistula was responsible for the lumen of the esophagus (Fig 1). In the other two patients (Nos. 1 and 8), an air lumen, which was located at the inferior level of the Blom-Singer voice prosthesis, was also observed (Fig 2).

Patients With Vocal Failure. Two patients (Nos. 2 and 7) complained of vocal failure after the achievement of a satisfactory voice following surgical resto-



Fig 2. Lateral views of magnetic resonance images of patients with satisfactory voice. Arrows — air lumen of esophagus, which was located at inferior level of fistula. A) Case 1. B) Case 8.



Fig 3. Polypoid soft tissues (arrows) inside fistula.

ration. Patients 2 and 7 failed to speak with the Blom-Singer voice prosthesis at 47 and 66 months after the laryngectomy with TEF, respectively. In both cases, a dislodgment of the prosthesis from the fistula was noticed before vocal failure. Granulation tissue around the fistula, at the side of the tracheostoma, was observed in case 2. Polypoid soft tissues could also be seen inside the fistula (Fig 3). The MRI of cases 2 and 7 demonstrated the fistula between the trachea and esophagus (Fig 4). An air lumen in the esophagus of case 2 was not detected by MRI (Fig 4A). Furthermore, the lower part of the fistula in case 2 showed a relatively hypertrophic structure in comparison to

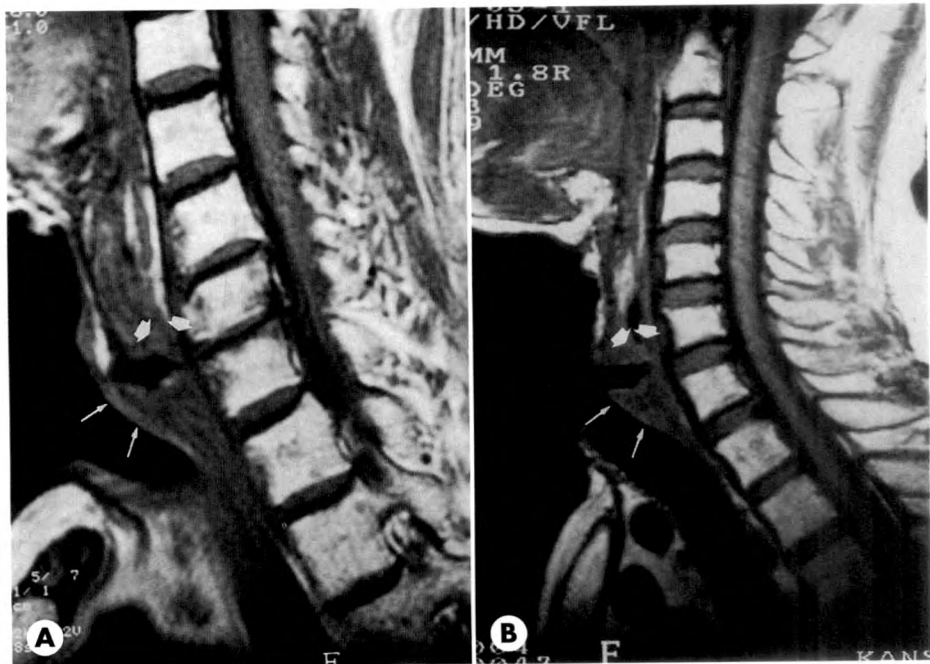
that in patients with satisfactory voice. Although an air lumen was detected in the upper part of the esophagus of case 7, it seemed to be interrupted by the bulging of soft tissues suggestive of granulation tissues (Fig 4B). The lower part of the fistula also showed the same thick structure as in case 2.

DISCUSSION

Tracheoesophageal fistula for vocal restoration is a rewarding operation. Phonation can be achieved by regular vibration of the esophagus due to expiratory airflow from the trachea passing through the inferior air port and the valve of the prosthesis.⁷⁻⁹ However, one of the long-term complications of TEF is the inability to successfully use tracheoesophageal speech, which can result from the appearance of granulation and polypoid tissues in the TEF tract.¹⁰ It has also been proposed that excessive digital pressure on the prosthesis for frequent speech is causal in the hypertrophic scarring of the posterior tracheal wall around the TEF site^{11,12}; thus, frequent use of a poorly fitting prosthesis may injure the posterior esophageal wall, resulting in edema of the esophageal wall and mucosal irregularities.¹³

In this study, the MRI findings in patients with vocal failure demonstrated a thick structure in the lower part of the TEF tract, as well as granulation tissues in the TEF tract located at the esophagus. The hypertrophy of the inferior TEF tract may be caused by mechanical irritation of the prosthesis against the inferior TEF tract, since the sharp edge of the inferior air port of the prosthesis may abrade the tract during speech. In contrast, no thick structures of the lower

Fig 4. Lateral views of magnetic resonance images of patients with vocal failure. Thick arrows — soft tissues suggesting granulation tissue in air lumen of esophagus, thin arrows — hypertrophic structures at lower part of fistula. A) Case 2. B) Case 7.



part of the TEF tract were observed in patients with a persistent satisfactory voice. The presence of granulation tissues in the TEF tract is considered to be the most common cause of the vocal failure, because it can block the airflow. In our experience, when granulation tissues exist, especially in the space of the esophagus, the dislodgment of the prosthesis or vocal failure can occur easily. Furthermore, when the granulation exists in the TEF tract, the prosthesis may not fit the TEF tract, resulting in spaces between the prosthesis and the TEF tract. These spaces can cause a salivary leakage from the esophagus to the trachea.

The use of MRI is not always necessary for the diagnosis of patients with vocal failure, because it is easy to find the affected part of the posterior tracheal wall around the TEF site in most patients by simple observation in the emergency room. However, in the case of patients who have had severe problems with multiple dislodgments of the prosthesis, the presence of granulation tissues at the TEF, and salivary leakage from the TEF, MRI can play an important role in confirming the origin and extent of the lesion, and can aid in evaluating the relationship of the TEF to the trachea and esophagus.

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IMAGING CASE STUDY OF THE MONTH

MAXILLARY SINUS APLASIA

DEAN M. CLERICO, MD

DANIEL J. GRABO, JR

KINGSTON, PENNSYLVANIA

INTRODUCTION

Complete aplasia or agenesis of the maxillary sinus is extremely rare, with only five reported cases in the world literature¹⁻⁴ and none in the English-language literature. Following is a case report of maxillary sinus aplasia, along with a discussion of the pertinent embryology and the ramifications of this finding to endoscopic sinus surgery.

CASE REPORT

A 66-year-old man complaining of postnasal drip and recurrent sinus infections presented to one of us (D.M.C.). In 1991, the patient had a septoplasty performed that did not significantly alter his symptoms. He reported no difficulties with his dentition or vision. On initial office nasal endoscopic examination, no gross pus or polyps were seen, although each middle meatus was narrow. A computed tomography scan of the sinuses was performed after 3 weeks of oral antibiotics and steroid nasal sprays. Along with patchy ethmoid mucosal thickening and a large concha bullosa on the

right, complete absence of both maxillary sinuses was noted (Fig 1). The patient underwent functional endoscopic ethmoidectomy, at which time complete absence of the uncinate processes was noted (Fig 2). His recovery was unremarkable and at 6-month follow-up he was asymptomatic.

DISCUSSION

The maxillary sinus is the first of the paranasal sinuses to develop in the human fetus.⁵ At the beginning of the third fetal month, a pocket or evagination of nasal mucosa develops in the middle meatus and serves as the future ostium of the maxillary sinus. This site of initial development is located on the inferolateral surface of the ethmoid infundibulum. At this stage, the uncinate process is composed of a thin rod of curved cartilage. Toward the end of the third fetal month, the ethmoid bulla chondrifies as an outgrowth of the lateral surface of the middle meatus. At about the fifth fetal month, the maxillary sinus begins to grow beyond the nasal capsule and into the spongy maxillary bone. This dates the time

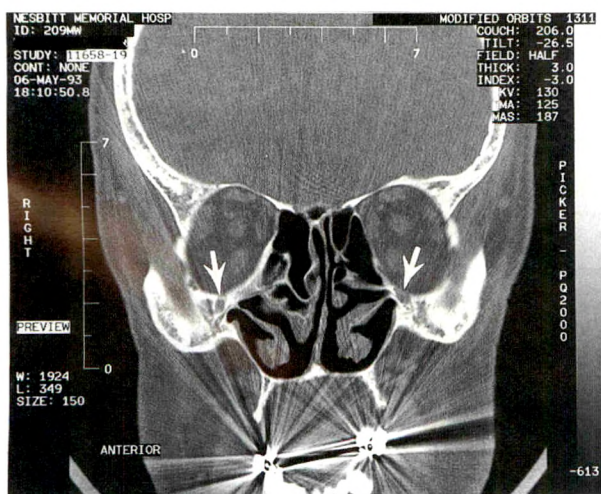


Fig 1. Coronal computed tomogram revealing complete agenesis of both maxillary sinuses. Inferior turbinate bone attaches directly to orbital floor. Arrows point to infraorbital nerves.

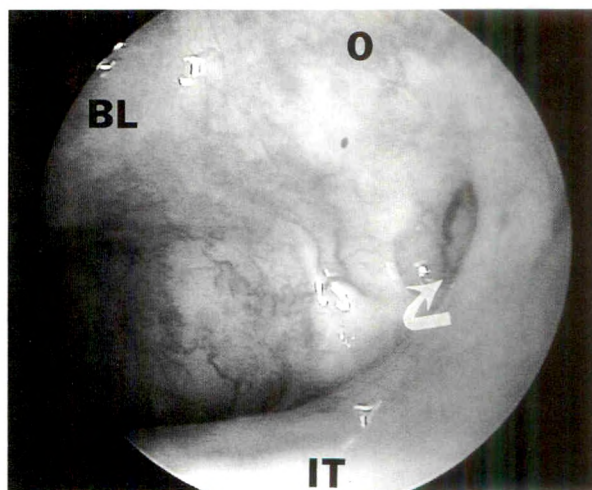


Fig 2. Endoscopic view of left middle meatus with 70° telescope. Small indentation (arrow) is only evidence of maxillary sinus. Complete absence of uncinate process is evident. O — medial orbital wall, BL — basal lamella of middle turbinate, IT — inferior turbinate.

From the Department of Otorhinolaryngology–Head and Neck Surgery, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania.
CORRESPONDENCE — Dean M. Clerico, MD, 534 Wyoming Ave, Kingston, PA 18704.

of disrupted development in our patient to between the third and fifth fetal months. Since his frontal and sphenoid sinuses were well developed, it can be surmised that either the intrauterine insult was temporary in nature, or the disruption was site-specific.

In the newborn, the maxillary sinus measures roughly 12 mm in width by 12.5 mm in height.⁶ Growth of the maxillary sinus in children and adolescents corresponds to the eruption patterns of both deciduous and permanent teeth.

The relation of the uncinate process to development

of the maxillary sinus has been pointed out by Bolger et al.⁷ In their material, hypoplasia of the uncinate process paralleled maxillary sinus hypoplasia. Not surprisingly, the uncinate process was completely absent in our case. Recognizing a hypoplastic or absent uncinate is important during endoscopic surgery, so as to avoid inadvertent entry into the orbit.

Complete aplasia of the frontal and sphenoid sinuses has been reported more frequently. Frontal sinus aplasia varies from 12% to 52%,⁶ depending on race. Sphenoid sinus aplasia has been reported in 1% to 1.5% of subjects.⁶

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CLINICOPATHOLOGICAL CONSULTATION

ROLE OF DNA MEASUREMENTS OF HEAD AND NECK TUMORS

CHRISTOPHER M. MILROY, MD, MRCPATH

SHEFFIELD, ENGLAND

KENNETH O. DEVANEY, MD

ANN ARBOR, MICHIGAN

ALFIO FERLITO, MD

PADUA, ITALY

ALESSANDRA RINALDO, MD

PADUA, ITALY

The measurement of DNA ploidy has been used as a tool to try to determine the prognosis of many neoplasms. DNA ploidy can be determined by flow cytometry or image analysis of a tumor. In squamous carcinomas of the head and neck, a poorer prognosis has been associated with nondiploid tumors. Similar results have been obtained from studies of salivary gland neoplasms. The role of DNA ploidy as a provider of independent information has yet to be determined. With rarer head and neck tumors, measurement of tumor ploidy has yet to be established as a valuable adjunct to routine light microscopic study.

INTRODUCTION

In trying to determine a tumor's behavior, pathologists have traditionally relied on such features as the general light microscopic appearance of the tumor, its histologic subtype, and the presence of lymphatic and vascular invasion. Newer techniques such as immunohistochemical markers are now being utilized in an attempt at predicting a tumor's behavior. Another established research technique, cellular DNA measurement, is now increasingly utilized to determine a tumor's clinical behavior. The measurement of the DNA content of tumors has been applied to many different tumors throughout the body and has been shown to provide prognostic information. In particular, DNA studies have shown that the ploidy status of ovarian and bladder tumors provides significant independent information.¹

CELL CYCLE

Cells divide by mitosis (except for resting sex cells) and they achieve this by going through the cell cycle. Cells not in the active cell cycle are described as quiescent or resting (G₀). Cells are recruited from this resting pool into the first stage of the cycle, known as G₁ (gap 1). This stage is followed by synthesis of DNA, referred to as the S phase. After DNA synthesis there follows a second gap phase (G₂), after which the cell undergoes mitosis, the M phase. Cells may then leave the active cell cycle, returning to G₀. Neoplastic cells go through the same cycle. Although it might be presumed that tumor cells have a shortened cycle, this is not always the case. When tumors are small, more cells are in the active cycle than resting, though as the tumor enlarges, cells

leave the cycle and return to the resting state. As tumor cells are synthesized, variable amounts of DNA may be present. The normal cell is diploid (2N), that is, has 46 chromosomes, except at the end of DNA synthesis before division, when the cell has twice the normal amount of DNA (tetraploid [4N]). Cell DNA measurements determine DNA ploidy, and correlation of ploidy and tumor behavior can be made.

MEASUREMENT OF PLOIDY

Two methods of measuring ploidy are available: flow cytometry and image analysis.¹ Flow cytometry uses tissue in which the nuclei have been labeled with a fluorochrome dye. The cells are disassociated from one another and then passed through a flow cytometer. At least 10,000 cells are necessary to perform the test and obtain reproducible results. The labeled nuclei are converted into an electronic pulse, the size of which is proportional to the DNA content of the nucleus. Control tissue is also used to establish a normal profile. From the data a histogram is generated. Peaks are formed for normal cells, the G₀ and G₁ phases (2N), with a second peak for cells in the G₂ and M phases (4N). The part of the histogram between the two peaks is the S phase. Normally, 85% to 90% of cells are diploid and 10% to 15% tetraploid. Tumors that peak at 2N are diploid, those that peak at 4N are tetraploid, and those outside these peaks are aneuploid. A tumor that has less DNA than normal is called hypodiploid, and a tumor that has a multiple ploidy (eg, 8N), polyploid.

By contrast, image analysis involves the use of histologic slides of the tumor that have been stained

From the Department of Forensic Pathology, University of Sheffield, Sheffield, England (Milroy), the Department of Otolaryngology, University of Padua, Padua, Italy (Ferlito, Rinaldo), and the Department of Pathology, University of Michigan, Ann Arbor, Michigan (Devaney).

CORRESPONDENCE — Alfio Ferlito, MD, Dept of Otolaryngology, University of Padua, Nuovo Policlinico, Via Giustiniani 2, 35128 Padua, Italy.

with the Feulgen technique. The integrated optical density of normal control cells and tumor cells is recorded via computer analysis. The normal cells set the 2N position. Only 200 tumor cells and 50 controls need to be measured to obtain sufficient data to generate a histogram of the ploidy profile.

The advantage of flow cytometry is the ease with which the data are produced. Image analysis is technically more time-consuming, though methods of automation will reduce this problem to some extent in the future. Image analysis has the advantage of permitting measurement of the tumor cells alone, while because flow cytometry requires larger amounts of tissue, nonmalignant tissue is also measured, along with necrotic material if present. Moreover, flow cytometry does not always produce a ploidy profile. Image analysis is ideally suited for small specimens. Measurement of tumor ploidy has been shown to provide independent prognostic information — for example, in transitional cell carcinoma of the bladder.¹

DNA PLOIDY OF HEAD AND NECK TUMORS

Squamous Cell Carcinoma. A number of studies have examined the relationship between DNA ploidy and prognosis. An overview of the flow cytometry studies of DNA in head and neck cancer has been given.² Most studies have shown a correlation between diploidy and better prognosis.^{3,4} Studies have tried to correlate outcome with response to chemotherapy.⁵ Some studies have shown a better outcome with aneuploid tumors. Ploidy studies may also play a role in determining a response to radiotherapy.⁶ Glottic tumors that are aneuploid are more radioresistant than diploid lesions and therefore should be treated primarily with surgery.⁷ El-Naggar et al⁸ studied 21 squamous cell carcinomas of the larynx by flow cytometry and found a high intratumoral heterogeneity of DNA content with consequent variations in prognostic implications.

Wang et al⁹ and Resnick et al¹⁰ found a correlation between aneuploidy and the presence of cervical node metastasis, respectively, in oral and laryngeal carcinomas. Cooke et al¹¹ have only noted a weak association between aneuploidy and incidence of cervical node metastasis in squamous cell carcinoma of the larynx. There are even some studies that have not demonstrated any correlation at all.¹²⁻¹⁴ The incidence of aneuploidy ranges from 29% to 80% in neoplasms of the head and neck.^{10,12,15-17}

Basaloid Squamous Cell Carcinoma. Luna et al¹⁸ have paradoxically found that patients affected by basaloid squamous cell carcinoma with aneuploidy

had a better mean survival time (39.5 months) than those with diploid tumors (16.3 months). DNA analysis of 10 cases of this aggressive head and neck tumor did not contribute any significant additional prognostic information.¹⁹

Neuroendocrine Neoplasms. Examination of DNA ploidy in series of laryngeal atypical carcinoids and small cell neuroendocrine carcinomas revealed tumors to be a mixture of diploid, tetraploid, and aneuploid lesions. No prognostic information was identified in this study.²⁰ Paragangliomas have been found to be both diploid and aneuploid.^{20,21} Aneuploidy in benign endocrine tumors is a well-recognized feature at other sites. Examination of a patient with multicentric paragangliomas found different ploidy results for different sites.²²

Salivary Gland Neoplasms. Studies have shown that there is an association between malignancy and ploidy, most salivary gland neoplasms being diploid.^{23,24} Aggressive malignant behavior has been correlated with aneuploid tumors. In a recent study both proliferative activity and nuclear DNA content in mucoepidermoid carcinoma correlated statistically with histocytologic grade and may be of assistance in predicting prognosis and survival.²⁵ Aneuploid adenoid cystic carcinomas correlate with poorer histologic grade,^{26,27} but in some tumors both diploid and aneuploid stem lines have been found.²⁸ Therefore, this heterogeneity observed in several tumors should be taken into consideration when using DNA flow cytometry as a prognostic factor.²⁸

Rhabdomyosarcomas. An examination of 13 adult primary head and neck rhabdomyosarcomas and 4 recurrent tumors found that 12 lesions were aneuploid, 5 neoplasms having more than one stem line.²⁹ One tumor was diploid. No correlation with behavior, histologic type, tumor site, or location was identified.

DISCUSSION

Measurements of the DNA content of tumors have shown that in some lesions, prognosis can be correlated to DNA content, though the same information can be obtained from other factors such as histologic grade. With rarer tumors the lack of large studies has not allowed full determination of the prognostic value of the DNA content of these neoplasms. DNA ploidy may have a role in determining a tumor's response to chemotherapy, though at present the value of measuring DNA content remains unclear and has yet to be proven in practical diagnostic pathology of head and neck tumors. Often the clinical significance of ploidy is controversial and confusing.

Historically, flow cytometry was initially adopted

as the standard means for determining the DNA content of solid tumors; this method suffers from the disadvantage that the tissue containing the neoplasm must first be disaggregated into individual cells that are subsequently analyzed. Clearly, this method does not allow the investigator to readily determine whether the flow cytometer is analyzing a pure population of tumor cells, a pure population of (benign) host cells, or — as is most often the case — an admixture of these two populations.

It is as a result of pathologists' dissatisfaction with the "blind" nature of traditional flow cytometric studies — that is, the observer was unable to be certain what proportion of the cells being studied was actually neoplastic — that attention has of late shifted to image analysis of histologic sections. Image analysis, while more labor-intensive than flow cytometry, allows the observer to correlate the analysis of DNA content with the light microscopic appearances of the

lesion; that is, image analysis permits the observer to select which specific group of cells within the (intact, not disaggregated) tissue specimen is not to be analyzed.

In general, results of DNA content analysis by flow cytometry and by image analysis correlate rather well with one another.³⁰ The two methods require the use of different equipment, however, and so it may not be practical for hospitals to invest in both systems for investigational purposes. As noted above, it remains to be seen whether DNA analysis of head and neck tumors will achieve significance in its own right as an independent prognostic indicator (separate, that is, from the information currently available from clinical staging and from routine pathologic ie, light microscopic, analysis). Until such time as it does, flow cytometry and image analysis will likely remain investigational tools employed by research facilities to study tumors of the head and neck area.

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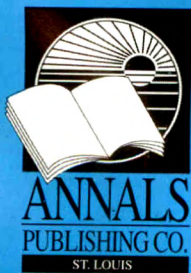
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